

A TEXT BOOK
OF HISTOLOGY

ARTHUR CLARKSON

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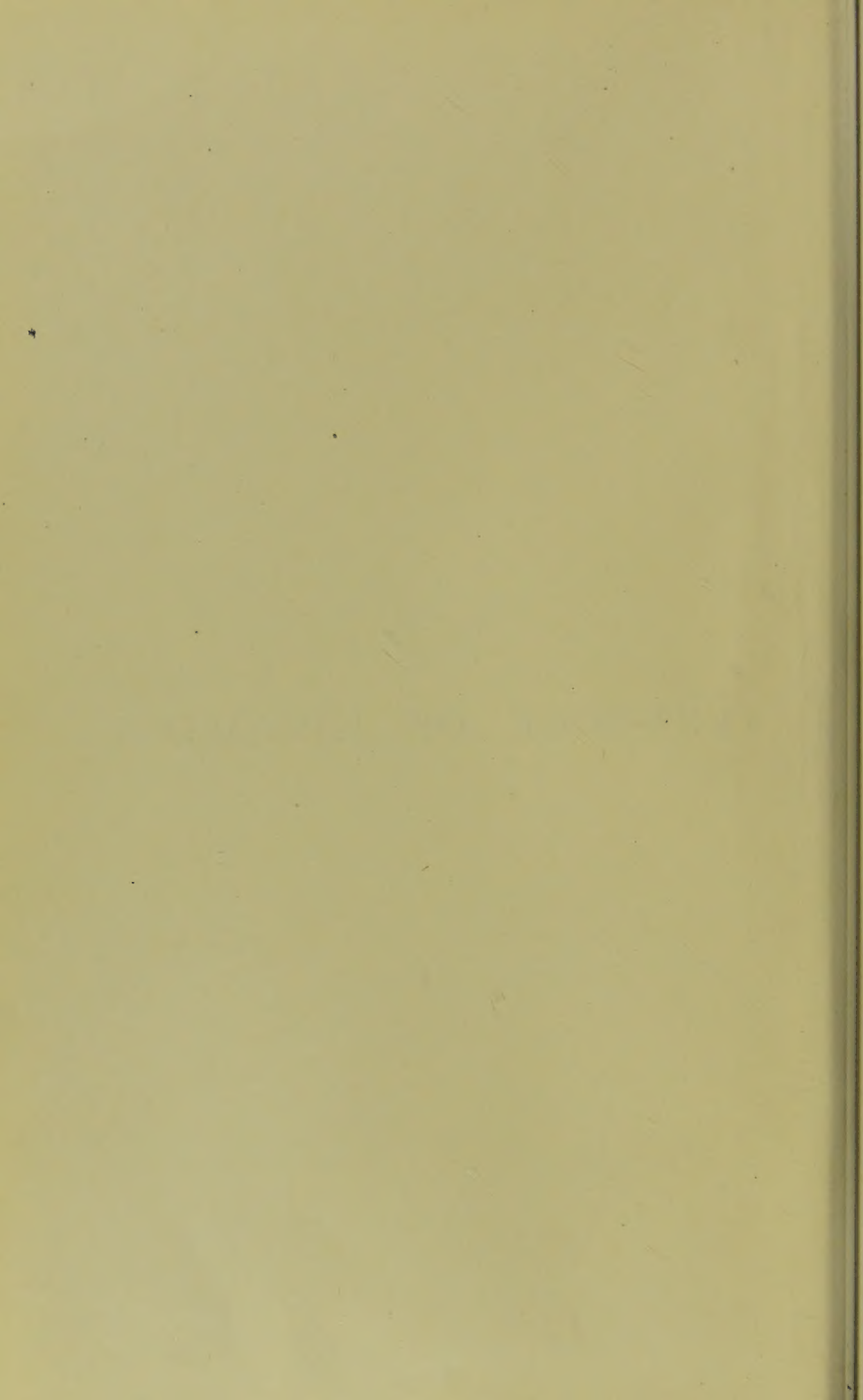
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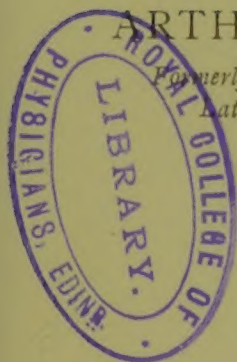
DESCRIPTIVE AND PRACTICAL.

FOR THE USE OF STUDENTS.

BY

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WITH 174 ORIGINAL COLOURED ILLUSTRATIONS.

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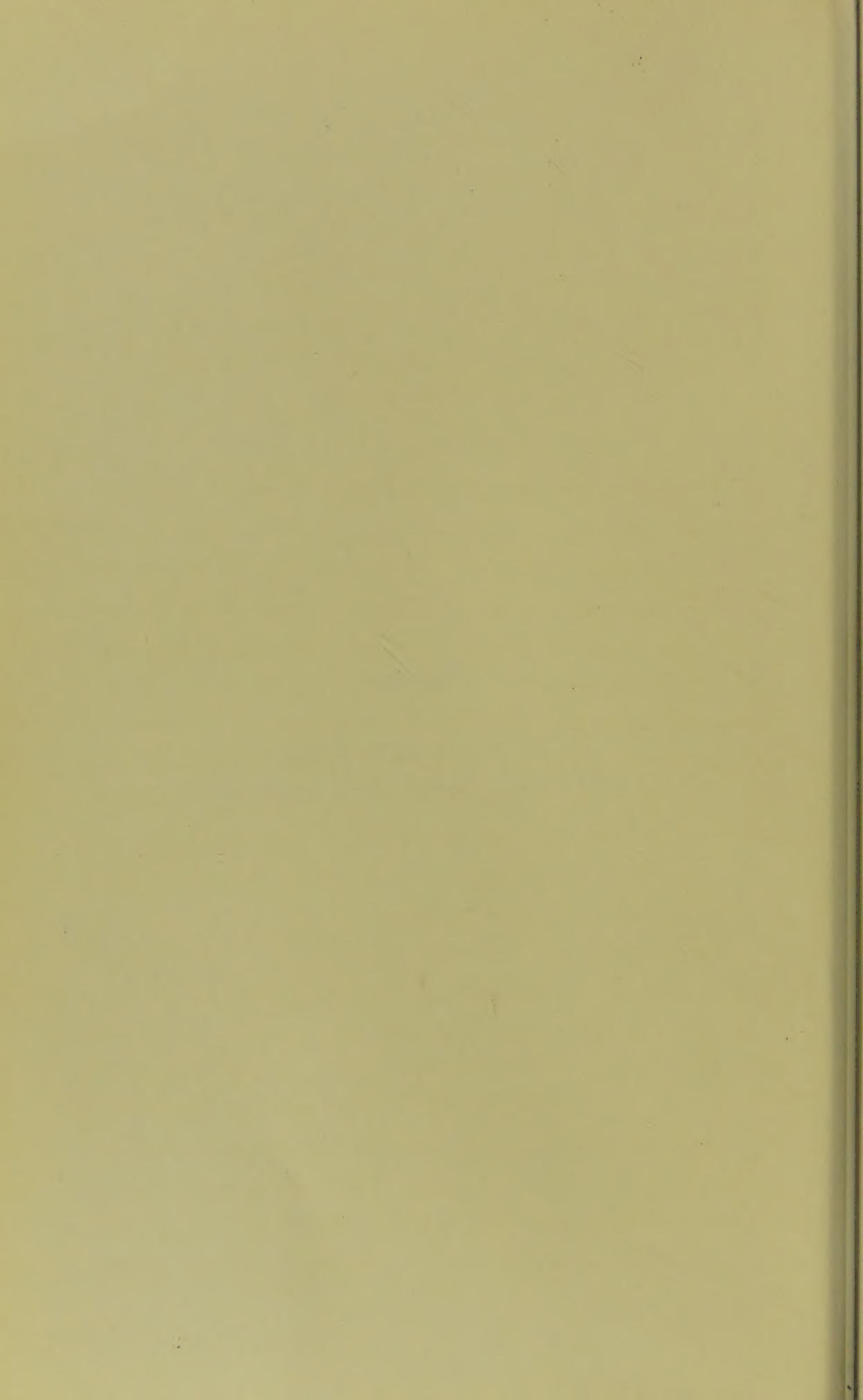
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DEDICATED
TO
THE MEMORY OF MY FATHER,
FREDERICK GEORGE CLARKSON, M.D.



PREFACE.

THE purpose of the writer in this work has been to furnish the student of Histology, in one volume, with both the descriptive and practical parts of the science. The working in of these together, presented, in the first instance, some little difficulty which, it is hoped, has been satisfactorily overcome. The first two chapters are devoted to the consideration of the general methods of Histology; subsequently, in each chapter, the structure of the tissue or organ is first systematically described, the student is then taken tutorially over the specimens illustrating it, and, finally, an appendix affords a short note of the methods of preparation.

It will be readily understood that a work of limited size, dealing with the descriptive and practical aspects of a large subject, cannot aspire to be of the nature of an exhaustive treatise on any part of it; and, in view of this necessary restriction, the writer has avoided, as much as possible, the discussion of disputed points, and contending views of only historical value. Nor has it been thought inadvisable to omit references almost entirely, as being really unnecessary to the understanding of the subject,

and chiefly of academic interest. In the practical part of the work also the same principle has been followed—only the well known and well tried methods are given; and, throughout, the object has been, while placing before the reader all that is necessary for his equipment as an Histologist, to avoid withdrawing his mind from the salient facts of the science by the introduction of a number of comparatively unimportant ones, of main interest to the specialist.

We seem, at present, to be rapidly approaching the dividing ways in the teaching of most sciences, and the question will soon be before us, Whether we shall be content to give our students a certain amount of real knowledge, or, in the endeavour to do more, perpetuate the reign of useless cram and practical ignorance.

The author would acknowledge his indebtedness generally to the current standard works on the subject; and especially to Professor Stirling's "Outlines of Histology" for many of the formulæ of re-agents.

He wishes also to avail himself of this opportunity to acknowledge his deep obligations to Professor Rutherford, of Edinburgh University, from whom, in his student days, he obtained his first insight into science and his love of it. From him also he learnt the art of constructing histological diagrams.

He thanks most cordially his friend, Professor Fawcett, of Bristol, for the trouble he has taken in reading the proof sheets, and for many valuable suggestions during the progress of the work.

Mr. J. J. Greenwood has kindly contributed the eight figures which bear his name, and Mr. J. W. Haigh *Fig. 38*.

He cannot express too strongly his appreciation of the kindness and courtesy he has received at the hands of his publishers, Messrs. J. Wright & Co., of Bristol, who have rendered him most efficient help throughout.

Finally, he would testify to the ability, zeal, and success with which Messrs. Maclagan & Cumming have executed the lithographic work.

ARTHUR CLARKSON.

Edinburgh, 1896.

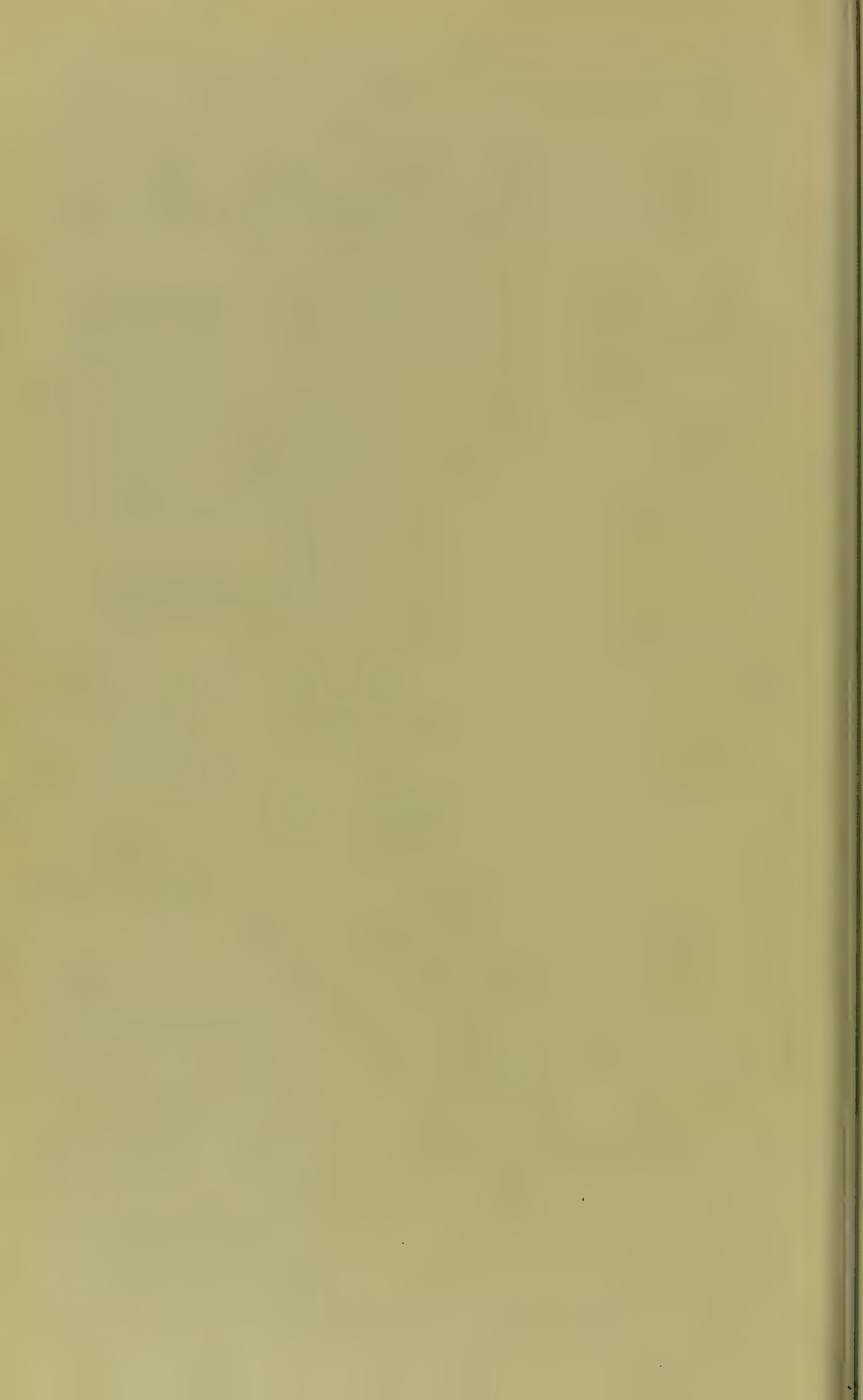


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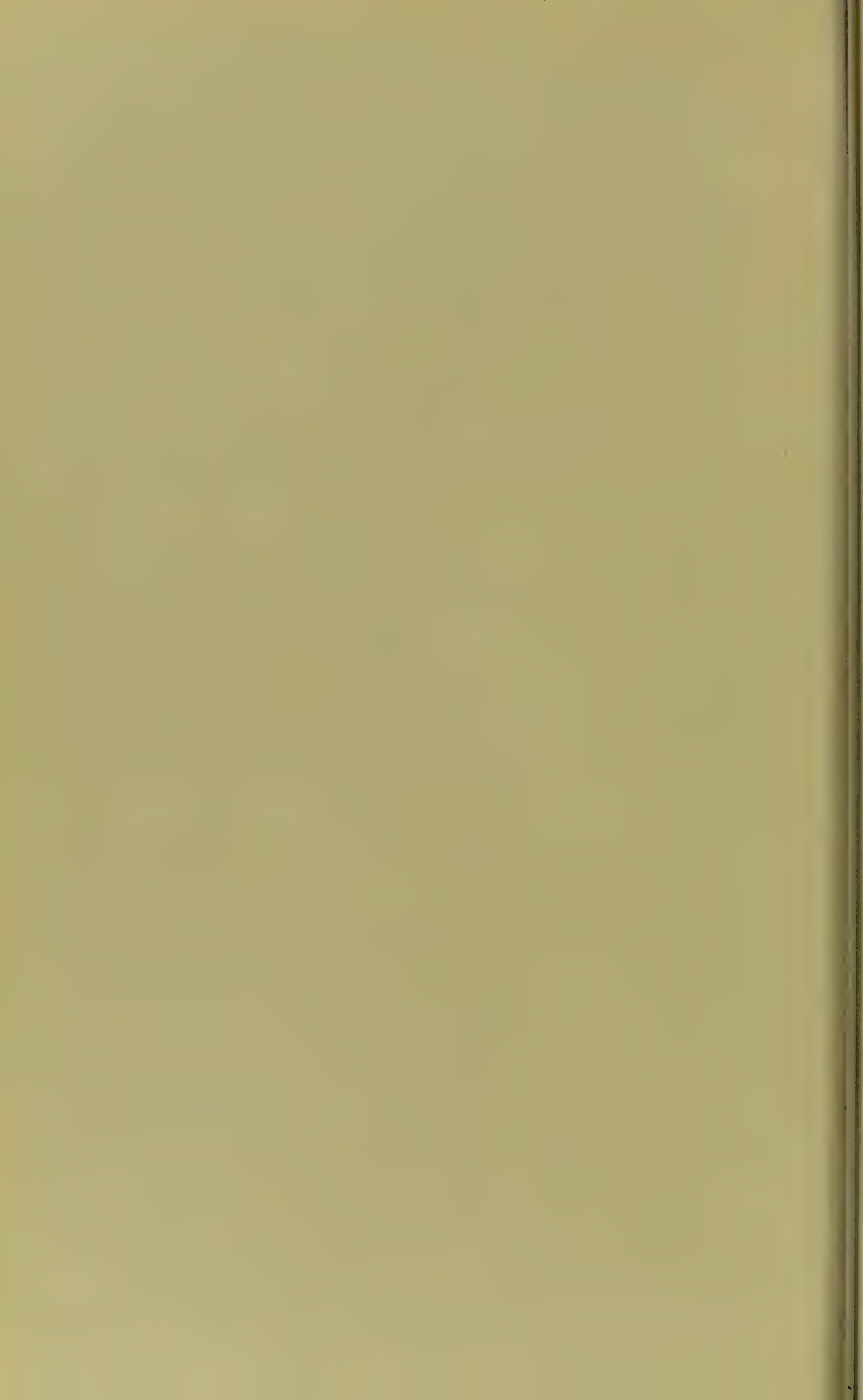
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A TEXT-BOOK OF HISTOLOGY.

CHAPTER I.

GENERAL METHODS OF HISTOLOGY.

HISTOLOGY treats of the structure of the body as seen under the microscope.

The structures of the body may be subdivided roughly into two great divisions:—

I.—THE SIMPLE TISSUES.

II.—THE ORGANS.

The Simple Tissues, as distinguished from the organs, are composed of comparatively few individual elements, and are found in various parts of the body. The Organs, on the other hand, are composed of a more or less elaborate combination of different simple tissues, and are definitely placed in some special locality.

Occupying a position midway between these two main divisions, there are certain "Systems" which are not composed of one simple tissue alone, and yet differ from the organs in that they are not restricted to one position but extend through the body generally. They differ from the organs, too, in the much smaller complexity of their formation.

The SIMPLE TISSUES of the body may be subdivided as follows:—

- 1.—*Cells Suspended in a Fluid.*
- 2.—*Cells Arranged on Free Surfaces.*
- 3.—*Cells Placed Interstitially in a Matrix.*

1.—**Cells suspended in a Fluid.**—The most noticeable example

of this is blood : where the cells—the blood corpuscles—float in a fluid, the liquor sanguinis.¹

2.—Cells arranged on Free Surfaces.—This includes the various epithelia, simple and stratified, in which the cells are in close apposition to each other, united by intercellular cement substance.

3.—Cells placed Interstitially in a Matrix.—Under this heading are included the various forms of connective tissue, and nerve and muscle tissue. In these the cell elements are to a greater or less extent separated from each other by the matrix in which they are embedded—a matrix which varies considerably according to the tissue.

THE "SYSTEMS."—The vascular and lymphatic systems are examples. An artery can scarcely be called one of the organs, but includes several simple tissues in its structure—epithelium, connective, muscular, and nervous tissue.

THE ORGANS.—The Lungs, Spleen, Liver, etc.

It is to be admitted that in such a classification there is a considerable amount of artificiality, and its adoption may be regarded more as a matter of convenience than scientific distinction.

All tissues are composed primarily of cells and intercellular substance. The intercellular substance may be very small in amount, as in the case of epithelium, where it is merely sufficient to cement the cells together ; or it may be very much greater, as in the case of hyaline cartilage, where it forms a homogeneous matrix in which the cells lie at some distance from each other. Again, the intercellular substance may have "fibres" of various kinds appearing in it, either as a result of "secretion" by the cells, or otherwise, as in the cases of ordinary connective tissue and elastic cartilage. The cells and fibres of any tissue, being the ultimate subdivisions of its anatomical structure, are termed the structural elements.

METHODS OF EXAMINATION.

The structures of the body may be examined in various ways.

I.—FLUID AND SEMI-FLUID STRUCTURES.

If, as in the case of blood, we are dealing with *cells suspended in a fluid* we may examine it :—

¹ The word "tissue" at first sight appears inapplicable to blood, but it is convenient to use the word in its widest possible sense. We speak of "mucous tissue," and the term is generally acceptable, though the intercellular substance can scarcely be regarded as a solid.

1.—*In the Fresh State.*

2.—*After Hardening and Staining.*

3.—*In the form of a Cover-Glass Preparation.*

1.—**In the Fresh State.**—A small drop of the fluid is placed upon the slide, and a cover glass applied to it, when it rapidly spreads to the edge of the cover, completely filling the space between it and the slide. Any superfluous fluid should be removed with bibulous paper, otherwise the slip is liable to float from its position if the stage of the microscope be slightly inclined.

After being examined thus, the specimen may be stained or subjected to the action of reagents by the method of irrigation. A drop of the staining fluid is placed at the side of the cover-slip touching its edge, and at the other is placed a small piece of bibulous paper. As the fluid beneath the cover passes into the bibulous paper on the one side, it leaves space for the entrance of the staining reagent on the other.

2.—**After Hardening and Staining.**—If, however, it be desired to make a permanent preparation, the above method will not suffice. The cells require “hardening” previously, *i.e.*, they require immersion in some fluid, which will so fix their present state, that they will not in a short time show evidence of physical alteration. Hayem’s fluid is very commonly employed for “fixing” blood corpuscles. The following is its composition :—

Sodic chloride	-	-	-	-	-	1	gramme
Sodic sulphate	-	-	-	-	-	5	grammes
Corrosive sublimate	-	-	-	-	-	0.5	grammes
Distilled water	-	-	-	-	-	200	c.c.

The blood is either run direct from the body of the animal, or after as short an interval as possible (*i.e.*, before coagulation), into an excess of the fluid (*i.e.*, 100 of fluid to 1 of blood). It is then stirred up with a glass rod so as to thoroughly mix the corpuscles with the hardening reagent. Twelve to twenty-four hours are required to complete the hardening process. The corpuscles should be stirred up frequently during this time. Afterwards the supernatant fluid may be decanted from the corpuscles, which will have settled to the bottom, and the vessel be refilled with water, in order to remove the superfluous salts. This process may be repeated more than once, in order that the corpuscles may be thoroughly washed. After filling the vessel

with water for this purpose, the corpuscles are stirred up with a glass rod and then allowed to settle to the bottom again before the fluid is decanted. If it is intended to stain the corpuscles, a weak solution of the reagent may now be added, and after again stirring them the whole may be left for a few hours. At the end of that time examine a drop of the fluid, taken with a pipette from the bottom of the glass. If the staining has taken place (and in the case of blood this is rapidly decided by noting the condition of the nucleus), pour off the supernatant fluid. The residue may now be treated in the following way, with a view to preservation. Pour melted glycerine jelly upon it, and stir up the whole rapidly with a glass rod, so that the corpuscles are evenly diffused through the jelly. Now transfer to a glass tube which has been in readiness; cork, and cool quickly under the tap. By this method of rapid cooling the corpuscles are retained scattered throughout the medium, which is more favourable to their preservation than if they are allowed to accumulate at the bottom of the tube. In this way blood, or other cells suspended in fluid, can be kept almost indefinitely, all that is required when preparations are to be made from it being that the tube should be placed in a vessel containing hot water, until the glycerine jelly melts; a glass rod is then dipped a little way into it, and a small drop placed upon a slide and covered. The jelly rapidly sets, and we have a permanent stained preparation.

3.—In the form of a Cover-Glass Preparation.—The method of preparation of a cover-glass specimen may be shortly described as follows: A small drop in the case of a fluid, or a scraping if of a semi-solid structure, is placed between two cover-glasses. These are gently pressed together between the thumb and forefinger, and allowed to slide on each other, with the object of causing a thin film to be spread out upon the surface of each. They are then separated by sliding one completely off the other, and are placed, film side upwards, on the table to dry. This takes a short time (varying with the temperature). The film on either cover may then be examined as it is, or after staining with some reagent such as methyl blue. The latter can be carried out in either of two ways. The cover slip may be placed film side downwards on the surface of a solution of the reagent in a watch glass; or a large drop of the solution may be placed on the film side of the slip, and, by means of a needle

or other instrument, smeared over its surface, care being taken that the film itself is not scratched. The slip is then left to stain for a variable time—in the case of methyl blue 1 to 2 minutes being sufficient. It is then seized with a pair of fine forceps, and gently laved in water to remove the superfluous stain. When all has been washed away, except that taken up by the film itself, the cover-glass is dried with slips of bibulous paper to as great an extent as possible without touching the film. If the glass be held with its plane vertical or sloping, a drop of water accumulates at its lower edge, from which point it is easily and safely removed. The non-film side can of course be completely dried. The cover-glass is now again left film side upwards to dry, and subsequently mounted in Canada balsam, or other mounting medium. This is done by either placing a small drop of the balsam on the film-side of the cover-slip and inverting it over the slide, or first placing the balsam on the slide, and then covering with the slip. Gentle pressure on the glass with a needle causes the drop to rapidly extend **between the apposed surfaces.**

The specimen, particularly when not stained, is sometimes mounted without the intervention of any medium. The cover slip is placed film downwards on a slide, and fixed in position by means of gummed paper.¹

II.—SOLID STRUCTURES.

The Solid Structures of the Body are for the most part examined in the form of sections. Sometimes, however, when the tissue takes the form of a thin membrane, such as the omentum, no cutting is required. The tissues may also be examined in a dissociated condition, *i.e.*, one in which their elements have been more or less separated from each other.

¹ (a.) Always handle the cover as much with forceps as possible in preference to holding it with the fingers.

(b.) See that the stained film is completely dry before mounting in balsam. If it is not, an emulsion will be formed by the mixture of the resin with the water.

(c.) The drying process may be quickened by moving the cover-slip, held by its edge in a pair of forceps, with the film-side uppermost, over a Bunsen burner, at some distance of course from the flame. It should not be allowed to get heated to much beyond the body temperature. When there is no occasion for hurry, it is safer to allow the film to dry naturally.

(d.) If at any stage of the process the student becomes doubtful as to which is the film side of the slip, he should draw the point of a needle over each. Only the film side shows the scratch caused by the removal of a line of material.

Dissociation of Tissues.—The process of dissociation is usually completed by teasing with needles on a slide. This may be all that is required in the case of fresh tissue, such as the nerve of a frog, in which the elements are readily separable from each other. In the case of many others, however, previous preparation is necessary, in order to weaken the coherence of their parts.

When a tissue is to be examined after teasing alone, it is, after removal from the body, placed upon a slide and teased with needles, either in a drop of the serum of the animal from which it has been taken, or some other neutral indifferent fluid. The object in using an indifferent fluid is that the tissue may not be altered in its histological characters. Either normal saline, or the serum of blood, is usually employed for this purpose; and of these two, the more generally used is the former, as it can readily be kept at hand. The strength of normal saline solution is 6 of sodium chloride (NaCl) to 100 of water (H_2O), or 6 in 1000.

After the tissue has been sufficiently teased to separate its constituent fibres from each other, as in the case of nerve and muscle, or to break up the cohesion between its individual gland cells, as in the case of such an organ as the liver, it may be examined after applying a cover-glass. If it be desired to subject it to the action of staining or other reagents, these can then be conveniently applied to it by the method of irrigation. Such a preparation is of course intended for immediate examination only.

If it be desired to make a permanent specimen, some method of previous hardening before dissociation is adopted. The piece of tissue may, for instance, be placed for a few hours in a weak solution ($\frac{1}{4}$ to $\frac{1}{2}$ per cent. of osmic acid) for this purpose, and at the end of that time transferred in bulk to some staining fluid, such as picro-carmin, in which it is kept till the stain has penetrated it; the length of time required depending on its size and porosity. Pieces of sciatic nerve of frog, or very small portions of liver, will require about twelve hours in a weak solution. A small fragment is then placed upon a slide, teased with needles, and mounted in glycerine or some other medium.

But, in many cases, mere teasing is not sufficient to dissociate the tissue elements from each other. They are bound together too closely either by connective tissue or cement substance,

which requires to be first dissolved. This is effected by immersion for a variable time in what is termed a "dissociating" fluid, of which the five following are those most commonly in use :—

1.—*Iodised Serum*, i.e., blood serum containing iodine in solution. A sufficient quantity of crystals of iodine should be dissolved to give the solution a light brown tint. Small pieces of tissue $\frac{1}{8}$ th of an inch in diameter require a day or two in this fluid before dissociation is attempted.

2.—*Dilute Alcohol* (alcohol 1 in 3). Ranvier's one-third alcohol is perhaps the most generally useful. It may be prepared by mixing one part of methylated spirit with two of water. In the case of small pieces of tissue $\frac{1}{16}$ to $\frac{1}{8}$ -in. in thickness, one or two days are sufficient. The time varies in the case of different tissues, some requiring a much longer period. The pieces are handled and examined from time to time.

3.—*Hydrochloric Acid* in water, varying in strength from .5 to 1.0 per cent., according to the rapidity with which it is required to act. This is useful for dissociating the convoluted tubules of the kidney from each other. Small pieces not more than $\frac{1}{8}$ -in. in thickness may be placed in different strengths of the solution, and examined from day to day, until they are of the requisite degree of looseness.

4.—*Caustic Potash* (of the strength of 1 of caustic potash to 3 of water) may be used for destroying the connective tissue between the fibres of unstriated and cardiac muscle, to facilitate the separation of the muscle fibres by teasing. It acts in a few minutes, and considerable care is required to avoid the destruction of the whole tissue. Water must not be added, or the muscle cells pass into solution.

5.—*Gastric and Pancreatic Digestion* are sometimes used for rendering elastic fibres (which are unaffected by them) more easily separable from each other, as in the case of ligamentum nuchæ of ox. Here we have to deal with a network of elastic fibres firmly bound together by ordinary white fibrous tissue, which becomes dissolved by the process.

After the tissue has been subjected sufficiently to the action of the dissociating fluid, it may be teased and examined as it is. It is, however, preferable to place it in bulk for twelve to twenty-four hours in $\frac{1}{2}$ per cent. of osmic acid, and afterwards to stain it in picro-carminic solution, or other reagent, before proceeding to

make a specimen from it. Elastic fibres need no treatment to ensure preservation.¹

When the tissue takes the form of a *thin membrane*, it may be examined fresh in normal saline, or after the addition of some staining or other reagent. If it be desired to make a permanent preparation, it is hardened previously; then stained (or the two processes may sometimes be conducted together) and mounted in glycerine, balsam, or other mounting medium.

Most tissues are, however, examined in the form of sections, cut by hand, or more usually in a microtome. Before cutting, nearly all tissues require "hardening." Some, such as bone and tooth, require softening; or these latter may require both, if the soft parts about them are to be preserved. When a tissue has been hardened or softened, as the case may be, and is ready for cutting, it requires to be "embedded" in some substance that will support it during the process. The tissue may be stained "in bulk" before being cut, or the sections may be stained afterwards. They are then mounted on a slide in some mounting medium.

In the succeeding pages, the tissue will be followed from the body of the animal through the successive stages of preparation up to the application of the cover-glass and the formation of a permanent specimen.

PREPARATION OF TISSUES FOR CUTTING.

An essential point in preparing tissues for section is to get them as fresh as possible, so that anything in the nature of retrogressive changes in the cells may be absolutely avoided. On this account other animals are, for the most part, used instead of man, as there is always more or less delay in obtaining anything from the *post mortem* room, the examination of the corpse being not usually made till many hours after death; neither can the organs be relied upon as being healthy.

The usual method of procedure when organs, such as the liver or kidneys of a small mammal, as the cat or rabbit, are required to be hardened, may be shortly described as follows:—

The animal is first killed, usually by placing it under a bell jar, just large enough to accommodate it, with a piece of tow soaked in chloroform or ether. When dead it is removed,

¹ For dissociation, as opposed to hardening, it is desirable to restrict the amount of fluid in which the tissue is placed.

and the abdomen thoroughly moistened with a wet sponge before making an incision, in order to avoid the inconvenience arising from the loose fur if the skin is cut without this precaution being taken. The abdominal wall may now be cut with blunt pointed scissors in the middle line from the sternum to just above the pubes, and again transversely about the level of the umbilicus. The contents of the cavity are next exposed by holding back the flaps, and the liver and kidneys are removed. These must then be moved rapidly to and fro in a basin of water, to remove the superfluous blood. Water not being an indifferent fluid quickly affects the tissues, so that this rough process of cleansing is made as short as possible. But in all cases where the tissue is such that contact with ordinary water will be injurious, normal saline should be used instead. The organs are now cut with a sharp scalpel or razor into suitable pieces, which in ordinary cases should be less than half an inch in their narrowest diameter. The smaller the pieces can be made, consistently with the sections subsequently derived from them demonstrating the structure of the organ, the better, as the penetration of the hardening reagent is the more rapid. It is sometimes convenient to cut the pieces rather large at first, re-cutting them more carefully the following day. Rapidly wash the pieces as soon as cut in water or normal saline, to again remove the blood, and place them in the fluid in which they are to be hardened. Of these fluids there is a considerable choice, and it depends greatly on what the organ is, as to which is selected. Some hardening reagents, however, may almost be termed general ones, that is to say, they are applicable to the treatment of nearly all tissues; whereas others, on the other hand, are only used for special purposes.

Whichever reagent is used, there are certain rules to be attended to in the process of hardening, which hold good for all. It is of primary importance that there should be an abundance of the fluid used; it should be largely in excess of the pieces of tissue placed in it, for if only sufficient to cover them in the vessel in which they are placed, it very soon loses its strength, and if not quickly replaced by fresh fluid, changes in the tissue rapidly supervene. On the second day the fluid, which will have become more or less turbid, should be poured off, the bottle containing the tissues rinsed out with water, and a fresh supply of the reagent added. It will be noted we are now

speaking of the commoner reagents, such as Müller's fluid, Müller and spirit, etc. After the second day, this should be changed as often as it becomes turbid. How often this occurs will depend largely upon the relative quantity of the fluid and the tissue—the greater the proportion of the former the less frequently will it require to be changed—and also on the nature of the tissue dealt with. Pieces of the lung require the fluid to be comparatively seldom changed, those of the liver more frequently. Roughly speaking, every third or fourth day would suffice in most cases, though as the hardening progressed, once in five, six, or more days, would be sufficient. The process usually occupies from three to five weeks, though in some cases a shorter time suffices. After hardening, the tissues are preserved in methylated spirit.

Various methods are adopted to ensure every piece being thoroughly exposed to the action of the reagent. To this end a layer of tow or cotton wool may be placed at the bottom of the vessel for them to rest upon. Sometimes it is convenient to suspend them separately by means of thread, the ends of the threads being attached to a glass rod crossing the mouth of the vessel; or they may be fixed in any other convenient way. As a general rule this is unnecessary if there is plenty of fluid, and too many pieces are not placed in the same vessel.

HARDENING REAGENTS.

The following are *hardening reagents* in common use:—

1.—**Alcohol.**—As a hardening reagent this is frequently used alone; it is also used to complete the process when it has been carried to a certain stage with some other reagent. When a tissue is to be hardened in alcohol alone, it should be employed in different strengths, commencing with a weak solution (50 per cent.), and passing by increases of 10 per cent. up to methylated spirit (95 per cent.), in which the tissues may be kept till they are required to be cut. One of its chief values lies in the fact that it is so readily obtainable in the form of methylated spirit. It is suitable for the preparation of almost any tissue if used in suitable strengths, except those in which it is required to retain the fat *in situ*, as in the case of the medullary sheath of nerve fibres. Here the fat—myelin—is dissolved out by alcohol.

Alcohol is found in the following different forms:—

- 1.—Absolute Alcohol, containing 96 to 99 per cent.
- 2.—Rectified Spirit " 84 "
- 3.—Methylated Spirit " 95 "

Except in special cases, methylated spirit is employed made up with water to strengths of 50 per cent., 60 per cent., 70 per cent., or 80 per cent.

Methylated spirit is also frequently employed as a constituent of a mixed hardening fluid (*see* Müller and Spirit).

2.—**Chromic Acid.**—This acid is used in strengths of from '2 to '5 per cent. It is rapid and powerful in action, with a tendency, however, to cause brittleness of the tissues. It requires a few days to work, the length of time depending on the tissue dealt with. Such as have been hardened in this fluid, and are subsequently preserved in alcohol, should be kept in the dark, as otherwise a deposit, which is difficult to remove, forms on the side of the glass. The same rule applies to all tissues which have been hardened in chromium salts. Before being transferred to alcohol, they should be thoroughly washed in water in order to remove the chromic acid or chromic salts.

Washing in water is most satisfactorily carried out by placing the tissues in a bowl, and allowing a small stream of tap water to run into it, until the washing is completed. Several hours at least should be allowed for this, and in many cases a day or two is not too much. The process is, of course, more rapidly carried out by the above method, than by simply placing the tissues in an excess of water, and changing it from time to time; as in the former case they are subjected during the whole time to a continuous supply of fresh fluid.

3.—**Chromic Acid and Spirit.**—This reagent is sometimes employed for rapid hardening, in the proportion of 2 parts of the '5 per cent. solution to 1 part of spirit.

4.—**Bichromate of Potassium.**—Of all hardening reagents this is the most useful. It penetrates the tissues evenly, and hardens them uniformly without making them brittle, as chromic acid is liable to do. It is used in strengths of from 2 to 5 per cent. Tissues after being hardened in bichromate, as in chromic acid, should be kept in the dark if they are preserved in spirit, after having been previously thoroughly washed in water. It takes about three weeks to harden most tissues. It is especially used for the central nervous system, but in this case the time

required may be doubled. Ammonium bichromate is used for the same purpose, but requires a longer time.

5.—Müller's Fluid.—This is the form in which bichromate of potassium is most usually employed, and this fluid constitutes the hardening reagent *par excellence*. There is really no tissue of the body which may not be prepared with it. It has the following composition :—

Potassium bichromate	-	-	-	25 grammes
Sodium sulphate	-	-	-	10 grammes
Water	-	-	-	1000 c.c.

The hardening occupies a more or less variable time, depending upon the porosity of the tissue and the size of the pieces dealt with. Generally speaking, from two to five weeks are required. At any period spirit may be added to hasten or complete the process.

6.—Müller's Fluid and Spirit.—This may be said generally to be composed of 3 parts of the former to 1 of the latter. As a matter of fact they are usually combined in any proportion that may be deemed advisable in the particular case. Sometimes only a little spirit is added to the Müller's fluid; sometimes they are mixed in equal proportions. The more spirit the more rapid the hardening. The mixture hardens tissues more rapidly than Müller's fluid alone.

7.—Pierie Acid.—This acid is sometimes used in the form of a saturated solution. Transfer the tissues by gradations, after hardening, to methylated spirit. More frequently it is employed in the following combination :—

8.—Kleinenberg's Solution.—

Saturated solution of picric acid	-	-	100 cc.
Commercial sulphuric acid	-	-	2 cc.
Filter : then add distilled water	-	-	300 c.c.

Kleinenberg's solution is especially adapted for the preparation of embryonic tissues. Transfer by gradations to methylated spirit.

9.—Chromo-acetic Acid.—

Chromic acid	-	-	-	2 to 2.5 grammes
Glacial acetic acid	-	-	-	1 c.c.
H ₂ O	-	-	-	1000 c.c.

Chromo-acetic acid is particularly useful for the study of nuclear changes. The pieces of tissue should be small, and only subjected to the action of the reagent for a day or two. They are afterwards washed in water and transferred by successive strength stages to methylated spirit.

10.—Osmic Acid.—In this acid we have an exceedingly useful, rapid hardener. It does not, however, penetrate deeply, and the pieces to be hardened should therefore be cut small. It may be used in strengths of $\frac{1}{4}$ to 1 per cent. It requires only a few hours to effect its purpose; in the case of thin membranes a still shorter time. The tissue may be washed in water and then transferred to spirit. Osmic acid stains all fatty material black. It is therefore especially used for staining adipose tissue and the myelin of the medullary sheath of nerve fibres.

11.—Chromo-aceto-osmic Acid (Flemming's Solution).—

Chromic acid (1 per cent.)	.	.	.	45 c.c.
Osmic acid (2 per cent.)	-	-	-	12 c.c.
Glacial acetic acid	-	-	-	3 c.c.

A weaker solution is sometimes used.

Like chromo-acetic acid, it is especially serviceable in the study of nuclear changes. It requires from a few hours to a day or more to act. The tissues are then thoroughly washed in water, and gradually transferred to strong spirit.

For the purpose of revealing the process of karyokinesis, in a specimen hardened with this reagent or with chromo-acetic acid, saffranin is the best stain.

12.—Corrosive Sublimite.—This may be used in either a watery or alcoholic saturated solution; the latter is the stronger. It hardens small pieces of tissue in from a half to two hours, the time required depending largely on the size of the piece of tissue. It is particularly useful for the rapid fixation of glands, when it may be advantageously employed in the form of a half saturated alcoholic solution: *i.e.*, to 50 c.c. of a saturated alcoholic solution add 50 c.c. of methylated spirit. The tissue should be transferred directly to alcohol to avoid precipitation of crystals of the salt.

13.—Silver Nitrate.—This salt is used both for hardening and staining, and will be more especially referred to under the heading of Staining Reagents.

The above list of "hardening" or "fixing" reagents is in no way intended to be a complete one. Only the commoner fluids are referred to, those in fact which are capable of some general application. Other methods of hardening, appropriate to special tissues, are referred to when the tissues themselves are under consideration.

With regard to the hardening of tissues generally, it is virtually impossible to lay down hard and fast rules as to the length of

time required in any reagent. In practice no such rules are followed. The tissue, after being cut into pieces of an appropriate size, and washed, is placed in an excess of the reagent, and its progress toward hardening is noted from day to day. Nothing but practical experience will enable the student to ascertain from the "feel" whether it is going on satisfactorily. A tissue ready for cutting gives, when handled, neither a sensation of softness nor hardness, but of a peculiar resilience, which becomes associated in the mind of the operator with ultimate success on the microtome.

Some of the more delicate tissues, of course, will not admit of handling, and in such cases, after the piece has been subjected to the action of the fluid for the average time, it may be supposed to be sufficiently hardened.

DECALCIFYING FLUIDS.

It is advisable always to harden the tissues before they are subjected to the action of the decalcifying fluid. They may be hardened in Müller's fluid, in Müller's fluid combined with spirit, or in spirit alone. Perhaps chromic and nitric fluid is most frequently used for the decalcification of bone and tooth. It has the following composition :—

Chromic acid	-	-	-	-	-	1 gramme
Water	-	-	-	-	-	200 c.c.
Nitric acid	-	-	-	-	-	2 c.c.

The time required for decalcification varies with the size of the piece of bone and the frequency with which the fluid is changed. From a few days to a fortnight is usually sufficient. The fluid should be changed as soon as it shows signs of darkening in colour. When this occurs it is a sign that it is losing its strength, which is due to the fact that the acid naturally becomes used up in the solution of the bone salts. It is easy to test the progress of decalcification from day to day, by thrusting a needle into the pieces, or by trying to cut them with a scalpel or razor. As long as any grittiness remains, the bone is not perfectly decalcified. After decalcification is complete, the pieces should be thoroughly washed in water to remove all the reagent, and transferred to the fluid (usually spirit) in which they are to be preserved.

EMBEDDING MEDIA.

- 1.—*Embedding in Gum.*
- 2.—*Embedding in Paraffin.*
- 3.—*Embedding in Celloidin.*

Before a tissue which has been hardened is ready for the microtome, it requires to be "embedded" in some material which will infiltrate its substance, and give it the necessary support during the process of cutting. Of the substances employed for the purpose of embedding, the following are the three most in use, viz., Gum, Paraffin, and Celloidin, each of which has its special advantages. The two most generally employed are gum and paraffin.

I.—Embedding in Gum.—The tissue to be cut must, on removal from the fluid in which it has been preserved, be thoroughly washed, by being placed in running water. It is particularly necessary that all alcohol should be removed, as its presence causes a precipitation of gum. Two to twenty-four hours' washing is required according to the size and density of the piece; only very small pieces with about $\frac{1}{8}$ th inch diameter will require so short a time as two hours.

After being washed in water, the tissue is transferred to the gum solution, which is usually a mixture of gum and syrup. It may be prepared as follows:—

Syrup	}	Cane sugar	.	.	.	28.5 grammes
		Water	-	.	.	30 c.c.
Gum	}	Gum acacia	.	.	.	57 grammes
		Water	-	.	.	310 c.c.

Take of

Syrup	-	4 parts	} Filter through muslin.
Gum	-	5 "	
Saturated solution of boracic acid	-	9 "	

The pieces are soaked in the above fluid, until it has thoroughly permeated them. Pieces $\frac{1}{2}$ in. square and larger are better left for at least twelve to twenty-four hours; if smaller, a shorter time will suffice. The process of infiltration is accelerated, if the gum containing the tissue be put in a somewhat warm place.

FREEZING AND CUTTING.

Microtomes.—After being thoroughly saturated with the gum solution, the tissue is ready for the next step in the preparation

of sections, *i.e.*, that of freezing and cutting. Sections are now almost invariably cut in a microtome, unless the tissue has been embedded in paraffin or celloidin, when they may be cut by hand, if desired. The latter method is, however, only available when a very few specimens are required. If sections are to be cut in any quantity, a microtome is essential.

Inasmuch as the mixture of gum and sugar is fluid at the ordinary temperature, it is necessary to freeze it before cutting; hence the microtomes used for cutting sections in gum are all of them freezing microtomes. Of these there are several varieties, but only the following three need be referred to.

Williams' Ice Freezing Microtome.—This consists of a round wooden box with a flat base and lid. From the centre of the bottom of the box a metal cylinder rises. The lid of the box is formed of a glass plate with a hole in the centre, through which the roughened disc-like extremity of the cylinder projects. The box possesses an outflow tube at its base, secured by a cork. When the microtome is to be used, it is carefully packed with a mixture of broken ice and salt, and the lid is clamped in position by means of a small screw. Now place the piece of tissue to be cut upon the metal disc. The process of freezing takes place more rapidly, if a small vessel, formed of some badly conducting substance, is inverted over the tissue and disc. Every few moments the vessel may be lifted up, and the progress of the freezing marked. It is initiated by an opaque whiteness occurring in the gum immediately in contact with the plate, and spreading up through the gum and tissue. It may be desirable, as the freezing advances, to add a drop or two more of the freezing mixture, as this should always extend an eighth of an inch at least beyond the tissue. This is conveniently done by dipping a pair of forceps, held closed, into the fluid from which the tissue was removed, and transferring the drop so obtained to the top of the mass on the freezing plate. The drop readily leaves the forceps if they are allowed to open in applying it. As soon as the opaqueness seems to have extended through the mass, it may be further tested as to its readiness for cutting, by touching it gently with the point of some instrument. A very little experience enables the operator to come to a conclusion as to whether the tissue is thoroughly frozen. Supposing this is the case, the sections are now to be cut. This is effected by means of a razor blade

fitted upon a "carrier," which consists of a metal framework moving upon three legs, and so contrived that by means of a milled head the razor may be either raised or lowered. The surface of the glass lid may now be moistened by smearing over it with the finger some of the gum and syrup solution. Place the carrier so that the edge of the knife is away from you and towards the tissue, and adjust it so that the razor edge will just remove a thin film from the surface of the frozen tissue. The carrier is now held by both hands, the milled head is slightly turned by the forefinger of the right-hand, so as to lower the blade, and the instrument is pushed forward along the glass plate, till a section has been removed from the mass. It is now retracted to its original position, the milled head again turned, and the carrier again pushed forward, so that the knife removes another section. This process is repeated until a considerable number of sections have accumulated upon the blade of the knife. These may now be transferred to a bowl of water, by wiping them off the blade with a camel's hair brush previously wetted. Any instrument, however, such as a needle in a handle, will do equally well. The sections are left in the water until all the gum has soaked out. This takes a variable time in different cases; usually two or three hours are sufficient. A single section gently moved to and fro in water parts with its gum much more rapidly.

After the sections have remained in the water for a sufficient time, they usually sink to the bottom of the bowl, and this may be taken as a sign that all the gum has been removed. They may now be lifted *en masse* with a needle, and transferred to a bowl of fresh water.

Swift's Ether Freezing Microtome.—This microtome only differs from the one previously described in being adapted for the application of ether as a freezing agent, instead of ice. It consists of a glass plate with a metal disc in the centre, on the under surface of which a spray of ether is directed. This spray may be worked by a hand-pump, or more conveniently by means of a bellows worked by the foot. A continuous stream of ether may thus be directed to the under surface of the plate during the process of cutting, leaving both the hands free for the manipulation of the knife carrier. Ether possesses this advantage over ice, that it is always on hand, and can be used with so little trouble in the way of preparing the microtome. Ice

is more convenient, however, if a great number of sections are to be cut, as, after the ice box is once packed, no further attention is required.

Ether has another decided advantage over ice as a freezing agent in that the degree of freezing can be so easily regulated. It frequently occurs, both in using ice and ether, that the tissue becomes over-frozen, that is, frozen so hard that it will not cut properly, and may even blunt the edge of the razor. When ether is employed this can be at once rectified by simply ceasing to work the pump, so that the spray ceases. The temperature of the plate with the tissue on it rapidly rises, the tissue readily cuts, and more cautious freezing can be recommenced. When ice is employed it is a different matter, as the freezing is necessarily continuous. The difficulty may be overcome, however, by dislodging the piece of tissue from the plate, allowing it to thaw, replacing it, and commencing to cut before it is again over-frozen. Other expedients, such as inverting a small heated vessel over the tissue to raise its temperature, will readily suggest themselves.

Cathcart's Ether Freezing Microtome.—This microtome differs from the foregoing in the nature of the glass plate and the knife used. Here the glass plate is represented by two parallel glass bars, between which is a movable metal disc, on which the tissue is placed. The disc is raised or lowered by a metal milled head placed underneath, and the sections are cut by means of a planing knife held in the right hand and passed along the bars. The ether spray, as before, plays on the under surface of the metal disc. In this case, as in the previous one, either a hand-pump or a bellows may be used. The chief advantage of this microtome is its cheapness. It is not suitable for the preparation of a large number of sections for class purposes, but it is perfectly efficacious for private work.

Both these microtomes are fixed to the edge of the table with a clamp.

STAINING.

The next step towards a permanent preparation is that of Staining. We will suppose first, that a considerable number of sections are to be stained at the same time. It is desirable to do this slowly with a weak solution. This is made by reducing some of the stock solution with water, the degree of dilution depending on the length of time allowed for the staining. The

diluted stain is placed in a small vessel, *e.g.*, of glass or porcelain, and the sections are immersed in it for several hours. The process may be watched by occasionally removing one from the rest with a needle, placing it in a bowl of water, and noting its colour. When this is sufficiently deep, all the sections are transferred to fresh water to remove the superfluous stain. They should be again transferred, and the action repeated, until they no longer colour the water in which they are placed.

When only one or two sections are to be stained the process may be effected much more rapidly. Prolonged staining in a weak solution is not always feasible. When it is desirable to stain a section in a few minutes, it should first be floated unstained upon a slide, then a drop or two of the reagent is placed upon it and allowed to remain for a few minutes. In this case, the ordinary solution is used and not a diluted one. If hæmatoxylin be used the section is, at the end of that time, floated off again with water, in order to get rid of the superfluous stain, and also to obtain the special colour effect which is thus produced (*see* "Hæmatoxylin"). The section is then again fixed upon the slide and mounted either in Farrant or balsam, as the case may be. If picro-carminé be used, it should not be washed off with water but the superfluous fluid removed with blotting paper, and the specimen mounted in Farrant's solution.

MOUNTING.

The sections may now be Mounted, either as they are in Farrant's solution (or Glycerine), or they may be "cleared up" with clove oil, and mounted in Canada balsam. The choice will depend on the degree of translucence required. In deciding this the following points are to be considered: (1,) The thickness of the section—the greater the thickness the more translucence will it bear; (2,) The depth of the staining and the stain used. Picro-carminé stained specimens are usually mounted in Farrant's solution; those stained with hæmatoxylin, in balsam. They may, however, if thin, and not deeply stained, be mounted in Farrant, and this is especially the case when definition under the high power is aimed at.

We will suppose a section has been stained with picro-carminé, transferred to a bowl of water, and is to be mounted in Farrant's solution. It has first to be placed in position upon the slide. This is done by "floating" it in the following way:—

Hold a clean slide by one extremity in the left hand and dip it obliquely into the water till three-fourths of its length are covered. With a needle held in the right hand gently move the section through the water, till it lies in the angle between the surface of the water and the upper surface of the slide. With the point of the needle fix the nearest corner of the section to the glass, and gently withdraw slide, section, and needle. If this withdrawal is successfully accomplished, the section will be evenly spread out in the centre of the slide. If not, it must be again dipped into the water, and the process repeated. A little practice renders this process of floating a section upon a slide a very simple one.

Inasmuch as water washes the picric acid out of a specimen stained with picro-carmin, it is usual at this stage to place on the section a drop of ordinary picro-carmin, and allow it to remain for a minute or two. At the end of that time remove the superfluous fluid with blotting paper, place on the section a drop of Farrant's solution, and "cover." It is not necessary to remove all the picro-carmin, for if a little remains, it diffuses itself through the mounting medium, and the tissue subsequently takes some of it up. Thus, specimens treated in this manner are usually more deeply stained after a few days than immediately after mounting.

The cover-glass is applied to the drop of Farrant's solution in the following way: Hold the cover-glass by its edges between the thumb and forefinger of the left hand; place it over the specimen, so that one edge rests on the glass a little to the left of the section, and the other on the point of a needle held in the right hand; allow the cover to gradually sink on the right side till it rests on the solution, controlling its subsidence with the needle. If the Farrant's solution is thick, a little gentle pressure on the surface of the cover-glass with the needle point may be required to make it spread out. Any superfluous Farrant may be removed with bibulous paper.

If the section has been stained with hæmatoxylin the same process may be repeated, with the exception that no additional staining is required after the section has been drawn on to the slide.

If, however, the specimen is to be cleared up in clove oil and mounted in balsam, the process is a little more complicated. The section must first be thoroughly dehydrated, which is

accomplished by means of absolute alcohol, as any water in its interstices will form an emulsion either with oil or resin. After it has been floated on the middle of the slide as before, the superfluous water is removed by means of bibulous paper or clean rag. A few drops of alcohol, applied either by means of a glass rod or from a drop bottle, are placed on the section and, after a moment or two, allowed to run off the slide by holding it at a slight inclination. Fresh alcohol is then applied and the process repeated two or three times until all the water has been removed. The superfluous alcohol is then taken up with blotting paper. It is not necessary, however, nor even desirable, to remove it all, as the section then runs a risk of having air enter its interstices while the small amount of alcohol left in it dries. Before this has time to occur place upon it a drop or two of clove oil and allow it to remain for about a minute, until in fact it has completely soaked through the tissue. A very little experience will enable the student to tell from the appearance of the section when this has taken place. Now remove the superfluous clove oil with bibulous paper, and apply Canada balsam. Cover in the usual way.

Sometimes, especially if the section is thick and time is required to obtain the full action of alcohol in dehydrating and clove oil in clearing, it is found more satisfactory to put the tissue through these stages in a watch glass. When this is the case the section, after being clarified in clove oil, is transferred from it to the slide upon the blade of a lifter, which is dipped beneath the section in the clove oil and then raised so as to remove it and some of the oil in which it floats. A little practice enables one to transfer the section to the slide without its becoming folded.

This process of clearing in oil and mounting in balsam almost invariably presents some initial difficulty to students. The whole secret of success lies, however, in thorough dehydration, and this is more important even for the balsam than for the clove oil. Often, when the latter appears to have acted satisfactorily, it is found that a white curdled opacity appears on the addition of the resin (balsam). In such a case it is usually waste of time to try to recover the section, as the precipitate is removed from it with great difficulty. It is far better to mount a fresh specimen.

Picro-carmin stained sections are not usually mounted in

balsam. They do not often require to be rendered very translucent, and the process of washing in alcohol removes, to a great extent, the picric acid.

Specimens mounted in Canada balsam require only to be labelled, the benzole in which the resin is dissolved evaporating from the edge of the cover-glass, which remains firmly fixed in position. Specimens mounted in Farrant's solution, however, require *ringing*, that is to say, that a ring of some cement substance should be laid round the edge of the cover-glass. It should be put on sufficiently thickly to ensure strength, and should extend at least $\frac{1}{16}$ of an inch both on the cover slip and the glass slide; making the breadth of the ring, roughly speaking, quite $\frac{1}{8}$ of an inch. Zinc white is as good a cement as any, but inasmuch as it does not readily "lie" on the surface of glass, its application should be preceded by that of a slightly broader ring of gold size. This also seems to greatly strengthen the ring of zinc white, which, if used alone, appears in time to suffer from the action of the Farrant's solution or glycerine. The ring of gold size takes a few hours to harden sufficiently to allow of the zinc white being applied.

Before the rings are applied, the Farrant at the edge of the cover-glass should have been allowed to dry, and a few days, at least, is requisite for this. In fact, it is well to leave the specimens for a week or more before proceeding to ring them. If no precaution of this kind is adopted, the cover-glass is so little fixed in position that it slips out of place under pressure of the brush. When a specimen is to be ringed, care should be taken to clear away any Farrant remaining at the edge of the cover slip with a damp cloth, and any moisture should afterwards be removed with a dry one. The slide is then fixed upon the plate of the turn-table by means of the clips. The centre of the cover-glass should be exactly over the centre of the plate. When the specimen has thus been "centred" as it is called, and while the plate is made to revolve, the gold size is laid on with a small paint brush. The brush is held in a fixed position with its point pressing lightly upon the edge of the cover. More than one brushful is usually required. Though the gold size will dry in a few hours sufficiently to admit of the zinc white being applied, it is better to wait at least a day; then apply the cement, remembering that this ring should be not quite so broad as that of the gold size beneath it.

II.—Embedding in Paraffin.—The method of embedding tissues in paraffin has many advantages over that of embedding them in gum. A far greater number of successful sections can be cut from a piece of tissue of equal size; the resulting sections are much more uniform; there is much greater control in the manipulation of the microtome; the sections can be cut very much thinner without falling to pieces; the tissue can be preserved in the blocks of paraffin for years, ready for cutting; the sections, when cut, can also be preserved indefinitely in boxes, before they are required to be mounted. It is, however, a somewhat more complicated process, and in the greater length of time required for it lies its comparative disadvantage. The process may be described as follows:—

We will suppose that the piece of tissue *e.g.*, liver, has been hardened in Müller's fluid, washed in water, and preserved in methylated spirit. The first step in the paraffin process is that of staining the tissue "in bulk," *i.e.*, before it is cut into sections. Borax-carminc and Kleinenberg's hæmatoxylin are both stains in common use for this purpose. A small piece of the tissue, about a $\frac{1}{4}$ inch cube, is placed in the solution in which it remains one, two, or three days, according to its size and penetrability. It is easy to determine when staining has taken place throughout the mass, by making an incision with a razor, and noting whether the colour has reached the more central part. If not, the tissue should be replaced in the fluid. When thoroughly stained it must, if the reagent be borax-carminc, be transferred to acid alcohol. This step is omitted in hæmatoxylin staining, but is necessary in the case of borax-carminc, on account of the diffusibility of the stain. That is to say, it stains not only the nuclei of the cells very deeply, but also the protoplasm around them, and the acid alcohol is required in order to lighten the depth of colour in the perinuclear portion of the cells. The length of time required will vary from a few hours to a day or two. If more than sufficient time is allowed, the process of "unstaining" proceeds too far, and finally the nuclei lose their colour. The acid alcohol becomes coloured with the stain as it is dissolved out of the tissue. On removal from the acid alcohol (or, if hæmatoxylin has been used, after removal from the staining fluid and washing in spirit) the tissue should be dried with a cloth, to get rid of as much moisture as possible, and transferred, if necessary, by

gradations in strength, to absolute alcohol, in which it is left until thorough dehydration has taken place. This will vary from a few hours to a day or more. The alcohol may require changing during the process, unless it is present in very considerable excess. After dehydration, again dry the pieces and transfer to turpentine, where they should remain from a few hours to a day or more until they are thoroughly saturated and ready for infiltration with paraffin. The turpentine should render the tissue more or less transparent, and the extent to which it has permeated may to some extent be judged by holding the piece up to the light. It may now, after drying with a cloth, be placed in the paraffin bath.

In the case of delicate tissues, however, it is not usually safe to transfer direct from turpentine to paraffin, and a mixture of the two may be employed as a transition stage. Or, the turpentine containing the tissue may be poured off until there is only sufficient left to cover it, and chips of paraffin added. The vessel is kept at a temperature sufficient to slowly melt the paraffin and, as it dissolves, more chips may be added at intervals, the principle being, to allow the tissue to become infiltrated with a solution of paraffin in turpentine, before it is subjected to paraffin alone. Finally, the tissue is transferred to unmixed paraffin.

A useful method in the treatment of embryos, or particularly delicate tissues, is the following: After dehydration with absolute alcohol, transfer to a mixture of one part benzol, and three parts alcohol; then to one of equal parts of each; then to one of three parts of benzol to one of alcohol; and finally, into benzol alone. The embryo may be kept in the mixtures from five minutes to half-an-hour for each stage, according to its size. When ready for embedding, pour off all but sufficient benzol to cover the embryo, add chips of paraffin, and place the vessel on a water-bath at a sufficient temperature to melt it. Add fresh paraffin from time to time as the benzol evaporates. At the end of a day or two all smell of benzol may have disappeared, and the tissue is in paraffin alone.

Different kinds of paraffin are recommended by different workers. The author has found that ordinary hard paraffin, with a melting point of about 60° c.c., has much to recommend it for most tissues. It cuts easily and evenly in the microtome,

and the sections can be preserved after cutting more readily than if paraffin with a lower melting point is used. In hot weather it is especially difficult to keep sections stored which have been cut in paraffin with a comparatively low melting point; the paraffin tends to melt, and the sections to become adherent to each other. Stirling, however, recommends a mixture of two parts of hard, with one of soft, paraffin.

Whether hard paraffin or a mixture of hard and soft be used, it should be kept at a temperature of about 1 degree above its melting point. It is easy by means of a temperature regulator and thermometer to ensure this, but even without the latter the same end may be roughly attained by keeping the main mass of the wax melted, and allowing a slight pellicle of congealed paraffin to remain on the surface. As long as this pellicle, however slight, is present, the temperature is not too high. The paraffin is kept in this state in small pans, which should be at least an inch in depth, and which fit into circular apertures in the lid of a water-bath. The tissue should be subjected to infiltration for at least a few hours, and in many cases a day or more is not too much; but if kept in melted paraffin too long it becomes hard, brittle, and contracted.

When the tissue is thoroughly infiltrated with the wax, the next step is to embed it in a solid block. This is done by placing it in a metal box (*Fig. A*), pouring melted paraffin over it and allowing the whole to cool. The box is formed in the

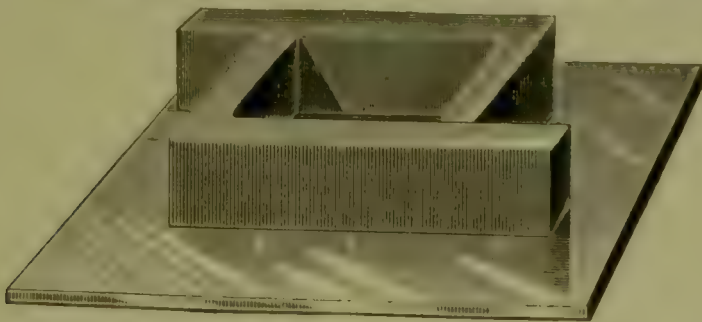


Fig. A. Embedding box.

following way: Two pieces of L-shaped metal are so placed in relation to each other that they form the four sides of a rectangle. The floor of the box is formed by a glass slide upon which the L's are laid. The arrangement will be readily understood by reference to the accompanying diagram. By altering the position of the L's, so that their short arms are

approximated or carried further apart, the size of the box may be diminished or increased.

Pour a little melted paraffin from the pan into the chamber—sufficient to cover its floor; now remove the piece of tissue from the pan either by inserting a needle into it, or by means of a pair of forceps, and place it on the surface of the paraffin in the box. Care should be taken to notice how the tissue is placed, with a view to cutting it in the requisite plane subsequently. The simplest way is to see that it lies so that the plane of the future sections is the same as that of the glass slide, or one of the sides of the box. Before the paraffin first poured in has time to congeal at the surface, add more from the pan, until the tissue is completely covered, and allow the whole to cool. If necessary, cooling may be much hastened by placing it in cold water, which must not, however, be done till the paraffin has set to a certain extent. When the wax is completely set, the slide and the pieces of metal may be readily separated from each other, and the block they contain. Sometimes the inner surfaces of the box are previously smeared with glycerine, but this is not absolutely necessary, as there is no real tendency on the part of the wax to adhere inconveniently, to either the metal or the glass, if they are clean. The paraffin block may now be “trimmed” before being fixed to the metal disc of the microtome. Trimming consists in cutting away with a knife superfluous wax from the front (the side to be cut from) and sides of the block. The base, as long as it is flat with the plane of the sections, may be left as it is, an excess of paraffin here being not disadvantageous. If the block is to be cut into “ribands” the rectangular shape of the sectional plane should be preserved, and $\frac{1}{16}$ -in. at least, of paraffin, should extend beyond the tissue on all sides; $\frac{1}{16}$ -in. is perhaps preferable.

It will be well here, to refer shortly to one or two of the microtomes most commonly in use for cutting sections in paraffin.

Cambridge rocking Microtome.—This is the most generally useful microtome. The razor is fixed, and the metal disc bearing the paraffin block is moved upwards and downwards against the edge by means of a horizontal bar, to the end of which it is attached. The end of the bar is raised and lowered by alternate movements, backwards and forwards, of a lever, connected with a toothed wheel. As the lever is drawn towards

the operator, the end of the bar bearing the tissue is raised above the edge of the razor, the toothed wheel is rotated through a segment of its circle, and the whole bar thereby projected, the thickness of a section, towards the knife. The thickness of the section depends on the extent of rotation of the toothed wheel, which is determined by means of a simple previous adjustment. As the lever is allowed to go back to its former position, a spring depresses the end of the bar bearing the tissue past the edge of the knife, which removes a section from it. When the next section is cut in the same way, its lower edge comes into contact with the upper edge of the first, to which it thus becomes adherent, and which it displaces in its position on the blade of the knife. As succeeding sections are cut, this adherence and displacement continues, until a riband of sections, in linear series, depends from the razor. The riband, however, when hard paraffin alone is used, is exceedingly apt to break, owing to the adhesion of the sections to each other not being sufficient to support its weight. To obviate this, the paraffin block, after being trimmed, may be dipped in melted soft paraffin. As soon as the film of soft paraffin which thus covers it, is set, remove it entirely from the front, and two lateral sides, so that only the upper and under surfaces are covered, when the sections are cut.

The metal "disc," in the case of paraffin microtomes, is detachable from the rest of the instrument, and the paraffin block is fixed to it before it is placed in position. In the Cambridge rocker, the disc is a paraffin one, fixed in the end of a short brass tube, the other extremity of which fits upon the end of the horizontal bar, upon which it can be moved backwards and forwards, to the extent of about an inch. The part of the tube containing the paraffin is separated from the rest by a metal septum. The paraffin is flush with the end of the tube, and to its surface the block to be cut is fixed. Hold the paraffin block in one hand, and the metal tube in the other; heat the surfaces to be joined in the outer part of the flame of a Bunsen burner or spirit lamp. As soon as the surfaces melt withdraw them from the flame, and press them quickly together; allow to cool, or hasten cooling by placing in cold water. The tube may then be fitted to the bar of the microtome, and so adjusted that the edge of the razor just shaves the surface of the block when it is brought past it, by raising and

lowering the end of the bar. The sides of the block, covered with soft paraffin, should look upwards and downwards. As the sections are cut they may be received upon a sheet of clean paper, placed below the razor, or directly into a card-board box. Card-board boxes, 3in. by 4in. by 1½in. deep, are very convenient for storing the sections when cut. They may thus be preserved almost indefinitely, if kept in a sufficiently cool place.

Minot's Microtome.—This is also a very excellent instrument for cutting sections in paraffin; but it is twice as costly as the "Cambridge rocker." The razor is fixed as before, and the tissue is moved upwards and downwards past it, upon a metal disc which, unlike the metal tube of the previous microtome, can be fixed in more than one plane, so that, should the paraffin block not be placed quite straightly upon it, a little adjustment rectifies the error. The instrument is worked by turning a wheel, similar to that of a sewing machine; each revolution moves a toothed disc through a segment of its circle, and thus approaches the tissue by the thickness of a section towards the knife. Previous adjustment decides what the thickness of the sections is to be; there are, however, only five or six thicknesses to choose from. (In the case of the "Cambridge rocker," this part of the instrument admits of much finer graduation.) There is included with this microtome a silk riband rotated round opposite bars by means of a milled head, which materially assists in the paying out of sections in series.

Thoma's Sledge Microtome.—For paraffin cutting this is also an excellent instrument, though less used than those already described. There are other microtomes, but the above are those in most general use.

Tissues embedded in paraffin may, however, be cut perfectly well by hand if only one or two sections are required, as is frequently the case in research. The paraffin block is suitably trimmed and held in the left hand, and thin sections shaved off its surface by means of a sharp razor held in the right. Of course, no uniformity in the sections, or the production of a "riband," is to be relied upon with this method.

After cutting, the first step towards mounting in balsam is usually that of *fixation* to the slide. This is essential in the case of thin specimens, which are liable to break up under the strain of the subsequent treatment, and also when sections are to be mounted in series. In this case, if they are not thus fixed

in position, they necessarily float out of their places when the turpentine necessary to remove the paraffin is applied. A film of *fixative* is laid upon the slide by means of a camel-hair brush, or it may be smeared over the required area with the tip of the finger.

This film should be as thin as possible, consistent with its fulfilling its purpose, as the coagulable element in it is in no way an advantage, and if present in any quantity may, by obscuring parts of the section, seriously impair its value. Even when lines of coagulated material are to be seen only round the section, they do not improve the appearance of the specimen. This disfiguration can almost, if not entirely, be avoided by taking care that the film is very thin.

The sections are then placed upon the slide in the position they are to occupy. It is sometimes desirable to slightly flatten them by very gentle pressure with the tip of the finger, but usually the next step is sufficient to effect the purpose. Hold the slide over the flame of a Bunsen burner or spirit lamp till the paraffin melts. This usually occurs quite sharply, as the necessary temperature is reached, the wax ceasing to be opaque, and the section, if not previously pressed, becoming flat with the slide. The paraffin should now be dissolved in turpentine. This may be effected by placing the slides on edge in a metal tray, which is lowered into a turpentine bath, in which they remain a short time; or by dipping the slide, held in the hand, several times into the bath; or thirdly, by pouring turpentine from a drop bottle upon the slide itself, and allowing it to run off again when the paraffin is dissolved. In working thus upon the slide itself, a second or third application of turpentine will be necessary to ensure all being removed. The latter method is the most rapid and simple when only one section is being dealt with; the two first methods, when many are being mounted on one slide. In any case the paraffin dissolves more readily if it is subjected to the action of turpentine before it has had time to set, after the slide has been heated over the flame of the Bunsen burner.

Several fixatives are in common use. A mixture of shellac and creasote, of the consistence of clove oil, is a very serviceable one; collodion and clove oil, albumen and glycerine, are also used.

When collodion and clove oil are used the latter should not

be employed for clarifying the sections. The paraffin should be removed with turpentine, and the specimen mounted directly in balsam.

Another method of fixation is also frequently employed. The sections are floated in ribands of suitable length upon the surface of warm water, when they become completely flattened out. The temperature should not be sufficiently high, however, to melt the soft paraffin which unites their edges, as the ribbons then fall to pieces. They are then floated into the required position on the slide, and the superfluous water removed with a clean rag or blotting paper. They are sometimes, now, laved carefully with spirit, the excess of which is subsequently removed in the same way, care being taken that the sections do not become displaced. They are then kept for several hours at a temperature of 50° c., and at the end of that time the slide is rapidly heated over a Bunsen flame to just beyond the melting point of the paraffin used. Turpentine is then applied as before. This method of fixing yields beautifully regular results, as the sections have less tendency to be folded, but it requires a little more care than when one of the fixatives is used. It is especially of service when the sections have become so folded that they could not otherwise be mounted. It possesses this advantage too, that we are not troubled with any remains of a fixative by which sometimes specimens are quite disfigured, particularly when egg albumen and glycerine have been employed.

After the paraffin has been removed, the sections may be mounted directly in Canada balsam, or may be previously subjected to clove oil. Its use has the advantage that any paraffin left inadvertently in the tissue, readily rises to the surface of the oil and may be poured off with its excess. Balsam is then added in the usual manner, and the specimen covered.

The above is a short description of the usual method of procedure, when tissue cut in paraffin has been previously stained in bulk. Sometimes, however, the sections are sufficiently thick to make the use of a fixative unnecessary, especially if they are not required to be mounted on the slide in series. In this case the fixative may be dispensed with; the section in paraffin being placed in position in the centre of the slide, and turpentine applied to it from a drop bottle. When all the paraffin has been dissolved out, the section may be clarified with clove oil and

mounted in balsam, or, as before stated, the clove oil may be omitted.

If it be desired to bring the sections into water before mounting, they are dealt with in the following manner. They are first placed in turpentine to remove the paraffin (if few, this process can be carried out in a watch glass, if many, in a larger vessel). It is advisable to have at least two turpentine baths, into which the sections are successively transferred, in order to ensure complete removal. They are then taken through baths of absolute alcohol in the same way, to remove the turpentine, and then, if sufficiently strong to stand the sudden transition, into water. If not, they must be taken through progressively weaker solutions of alcohol, say 70 per cent. and 35 per cent. before reaching water. After this, if they are to be mounted in balsam, they are floated upon the slide, again dehydrated, cleared with clove oil, and mounted in the usual manner. In this case, of course, the clarifying oil is not dispensed with, as the section is not in turpentine, which is itself a clarifying reagent (*see* "Clarifying Reagents"). When the tissue has not previously been stained in bulk, much the same process requires to be gone through, but the sections must be stained. This may be done either before or after they are placed upon the slide. If before, they are transferred from water to the stain in a watch glass or other small vessel, and then back to water again, when they are mounted as before. If after, the staining is carried out previous to the process of dehydration, or previous to the application of Farrant.

III.—Embedding in Celloidin.—The third method, that of embedding in celloidin, is particularly applicable to those tissues which too readily fall to pieces when in the form of sections, to render either the gum or paraffin process available. The cochlea is a good example. It is almost impossible to rely on obtaining a satisfactory specimen of the organ of Corti, unless the tissue is cut in celloidin. The advantage of it lies in this, that it is not necessary to remove it when the section is mounted. Cutting in gum is totally out of the question in such a case. Paraffin is certainly more feasible, but the section, if cut thin, almost invariably goes to the bad, when the wax is dissolved out with turpentine.

Celloidin is a form of intro-cellulose or pyroxylin. It is best used in the form of cuttings; the following is the method:—

Place the tissue to be embedded, in absolute alcohol, till it is

thoroughly dehydrated, and then transfer to a mixture of equal parts of alcohol and ether for twenty-four to forty-eight hours.

Two solutions of celloidin are commonly used. They are both prepared by dissolving the cuttings in a mixture of equal parts of alcohol and ether; the first is of a distinctly thin consistence, while the second is as distinctly syrupy.

After remaining in the mixture of alcohol and ether for a day or two, the tissue is transferred to the dilute solution of celloidin, in which it may be left for a similar length of time, or until complete saturation has taken place. It is then placed

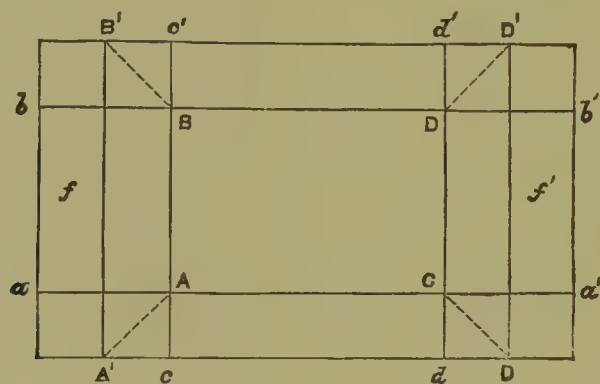


Fig. B. Plan of embedding box.

in the stronger solution for several days. The tissue, with celloidin in sufficient quantity to cover it, is now transferred to a paper embedding box (*Fig. B*). The following directions for making such a box are taken from Prof. Stirling's excellent work, and will be readily followed.

"The paper is first folded along the lines aa^1 and bb^1 , then along cc^1 and dd^1 , always folding the paper towards the same side. The diagonals AA^1-DD^1 and BB^1-CD , are indented by means of the point of a lead pencil, or the paper is folded along these lines. These corners are then bent up between the finger and thumb, and then bent round, so as to be applied to the sides AB and CD of the oblong, and are fixed there by turning down the flaps ff^1 ." A pill box may also be used, if preferred.

Place the tissue in one of these boxes, the sides of which may be previously moistened with alcohol, and cover it with the celloidin from which it has been removed. Allow it to remain thus for half to one hour, that is, until by evaporation of the alcohol and ether, the celloidin begins to harden on the surface. Then transfer the box and its contents to 80 per cent alcohol, in which it

must remain for a day at least, sometimes for two or three. When sufficiently hardened to cut, somewhat like soft paraffin, it may be removed, the paper box stripped away, and the celloidin block preserved in 70 per cent. alcohol.

To cut sections, the celloidin block, after trimming with a knife to the required size, should be embedded in gum. To this end the alcohol must first be removed, by placing it in running water for several hours, after which it is transferred to the usual freezing mixture. When the gum has infiltrated the mass, it may be placed on the plate of one of the microtomes described, and sections cut in the usual manner. The knife should be moistened with 70 per cent. alcohol. After cutting, the sections are transferred to water, then stained, and mounted in glycerine or balsam. When they are to be mounted in balsam, however, neither absolute alcohol nor clove oil must be used in the process, as both dissolve celloidin; 95 per cent. alcohol must be used for dehydration, and origanum or bergamot oil, or creasote, for clarification.

GENERAL REMARKS.

It will be seen from the above description of the various methods of hardening, embedding, cutting, etc., that there is a good deal of choice, not only as to the general method adopted, but also as to the details of the process in its many stages. The student cannot be too earnestly recommended, to make himself thoroughly conversant with one plan of action, before proceeding to the practice of another. It is always to be borne steadily in mind, that the object to be attained, is to be able from a given piece of fresh tissue, to prepare a successful permanent preparation. When the student is able to do this practically for himself, by a method with which he is thoroughly familiar, he has accomplished a great deal; more in fact, at the present time, than most of his fellows have. Nothing is to be more deprecated than what may be termed an examination knowledge of many methods, and a real knowledge of none. As a mere question of labour required, also, it will be found in the end, that a practical knowledge of a few methods is far more easily obtainable than an encyclopædic one of all. Again, it is impossible from a book description to understand the processes. All the various stages are liable to so much modifica-

tion to suit special cases, so much depends on the size, delicacy, and porosity of the tissues, the strength of the staining solutions, the temperature at which the process is carried out at any particular stage, the time at the operator's disposal, etc., that no universally applicable rules can be laid down, and the task of committing to memory, from a book, the various possible contingencies, is as herculean as it is useless.

The student is advised to acquire his experience in somewhat the following order.

First, learn to mount a specimen (already stained) from water, in (a) Farrant's solution, (b) Canada balsam.

The "*pons asinorum*" of the histological tyro is usually found in the difficulties of the latter process. Many sections are usually destroyed, before he realises that water and resin will not mix satisfactorily, and that dehydration with absolute alcohol must be complete.

Next, the process of staining a section, before mounting, in picro-carmin and hæmatoxylin, should be practised as directed, the picro-carmin specimen being mounted in Farrant's solution, one stained with hæmatoxylin in Farrant and another in balsam. After mounting, remove the superfluous mounting fluid (if any), from the slides with a piece of rag, label, and set on one side. The Farrant mounted specimens will require ringing, after the gum at the edge of the cover-glass has had time to dry.

The process of embedding tissues, already hardened and preserved in spirit, in gum, and cutting sections in a freezing microtome, may now be practised, and after that the student should make himself thoroughly conversant with one of the methods of hardening fresh tissues, say in Müller's fluid or Müller's fluid and spirit.

It is assumed, of course, that the student is working in a laboratory; if he is commencing *ab initio* by himself, he will reverse the order given above.

When the above processes are completely mastered, the paraffin method should next be taken in hand. This may be advantageously dealt with in the same way. First, become acquainted with the process of mounting single sections already stained, (a) with the help of a fixative, (b) without, in both cases dissolving the paraffin with turpentine, on the slide, and mounting in balsam. Then practise getting rid of the paraffin, and transferring to water before mounting, both with stained and

unstained specimens, the latter requiring to be stained in the same way as sections cut in gum.

Next, starting with a piece of tissue already preserved in spirit, put it through the various stages of staining, with borax-carmin, decolorising with acid alcohol, dehydrating with absolute alcohol, infiltrating with turpentine and paraffin, and finally cut it in the Cambridge rocker or Minot's microtome.

Now practise embedding tissues, previously prepared, in celloidin, cutting, staining, and mounting them as directed.

When these methods of gum, paraffin, and celloidin, are in principle thoroughly understood, the student may with advantage practise as many variations, in the way of staining, clearing, mounting, etc., as he may think fit. A short account of these is given in the succeeding chapter.

CHAPTER II.

GENERAL METHODS OF HISTOLOGY (*Continued*).STAINING, CLEARING, MOUNTING:
INJECTION OF BLOOD VESSELS.

STAINING REAGENTS AND METHODS.

TISSUES, as before stated, may be stained in bulk, or after they are cut into sections.

Some reagents may be termed *General Stains*, *i.e.*, they stain to some extent all or most of the elements of the tissues. Of these, some are especially nuclear stains, that is to say, they stain the nuclei of the tissue deeply, and the cell protoplasm and intercellular substance less deeply; others are more diffuse in their action and stain the whole more evenly, the tissue thus requiring partial decolorisation after staining. On the other hand some reagents may be termed *Special Stains*, *i.e.*, they stain some particular substance, such as the medullary sheath of nerve fibres, the interstitial cement substance, etc. Again, two or more stains may be combined to form a *Compound Stain*, or sections may be stained successively with each. The following scheme gives examples of these three subdivisions:—

<i>General Stains</i>	$\left\{ \begin{array}{l} (a,) \text{ Nuclear} \\ (b,) \text{ Diffuse} \end{array} \right.$	$\left\{ \begin{array}{l} \text{Picro-carminc} \\ \text{Hæmatoxylin} \\ \text{Borax-carminc} \end{array} \right.$
<i>Special Stains</i>	$\left\{ \begin{array}{l} \text{Osmic acid} \\ \text{Nitrate of Silver} \\ \text{Chloride of Gold} \end{array} \right.$	
<i>Compound Stains</i>	$\left\{ \begin{array}{l} \text{Picro-carminc} \\ \text{Eosin-hæmatoxylin} \end{array} \right.$	

For convenience the chief staining reagents will be considered under the following headings:—(A,) Carmine, Hæmatoxylin and Eosin; (B,) Aniline; (C,) Metallic; (D,) Compound.

A.—CARMINE, HÆMATOXYLIN AND EOSIN STAINS.

I.—*Carminé.*

Picro-carminé is one of the most generally employed stains we have. It is applicable to any tissue, and possesses this advantage over hæmatoxylin, that sections stained with it improve with keeping; whereas, those stained with hæmatoxylin, are very liable to fade in colour. It is also a double stain.

Nuclei and the white fibres of connective tissue are stained with the carminé; the former much the more deeply. Elastic fibres, superficial epidermis, nail and hair (corneous portion), are stained with the picric acid; perinuclear protoplasm and muscle fibres are affected by both in varying degrees.

If the picro-carminé be used before floating on the slide, the sections require additional staining afterwards, as the picric acid is washed out with water, but if stained on the slide itself the surplus picro-carminé is removed with bibulous paper, leaving the section still soaked with a certain amount. A drop of Farrant's solution is then applied, and the specimen covered.

If the section is to be mounted in balsam, however, the alcohol used for dehydration and the clarifying agent should both contain picric acid.

Picro-carminé is prepared as follows (Ranvier):—Make a saturated ammoniacal solution of carminé: 4 grains of carminé are mixed in a mortar, with a little water and 10 cubic centimetres of liq. ammon. fort. added. Mix, and add 200 c.c. water, allow to stand for 24 hours, filter, add thymol for preservation, and store in a bottle. The solution should be distinctly alkaline. If strongly alkaline, the stopper may be left partially out till the smell of ammonia is weaker. Add the above to a saturated solution of picric acid till precipitation occurs, stirring the while with a glass rod. Expose the mixture in a large porcelain dish, covered with a glass sheet. It is left thus to crystallise and evaporate, till its bulk is reduced to one third. Then decant the supernatant fluid, filter, and evaporate to dryness on a sand bath. Dissolve the residue and the first crystalline deposit in water, and again dry. Pound the residue in a mortar, and redissolve it, in the proportion of 1 gram.

of the powder to 100 c.c. of water. The following is also a useful formula :—

Saturated solution of picric acid	- - - - -	200 c.c.
Ammoniacal solution of carmine	$\left\{ \begin{array}{l} 1 \text{ gram. carmine} \\ 3 \text{ c.c. water} \\ 3 \text{ c.c. strong ammon.} \end{array} \right\}$	6 c.c.

Mix and expose in a shallow dish, protected from dust, to evaporate to half, or one third its bulk, filter, add thymol for preservation.

Alum-carminc.—Like picro-carminc this is a nuclear stain. It may be prepared as follows (Grenacher):—Add 1 gram. of powdered carminc to 100 c.c. of 5 per cent. solution of ammonia alum, and boil; filter when cool. Add thymol. Sections may be left in this fluid a considerable time, without over-staining, or staining too diffusely.

Picro-lithium carminc.—Dissolve 2.5 grams. of carminc in 100 c.c. saturated solution of lithium carbonate, add 200 c.c. saturated solution of picric acid. The stain is diffuse and acts rapidly. Sections after staining require immersion in acid alcohol.

Carminc.—The strong ammoniaal solution of carminc described above may be kept in stock, and diluted according to the strength required. The solution should be filtered before using, in order to remove any precipitate which may have fallen from over-evaporation of the ammonia.

Borax-carminc.—Borax-carminc is an extremely useful reagent for staining tissues in bulk, *i.e.*, before they are cut into sections, especially for those which are to be cut in paraffin. It is one of the diffuse stains, however, colouring all the elements of the tissue too uniformly. In order, therefore, to use it as a nuclear stain, place the piece of tissue in acid alcohol, which will dissolve out the stain to a great extent, from all but the nuclei of the cells, which remain deeply coloured. The tissue should not, however, remain in the alcohol too long. For pieces $\frac{1}{2}$ -in. in diameter, a few days may be required for the action of the borax-carminc, and twenty-four to forty-eight hours for the acid alcohol. It is easy to watch the progress, by making cuts into the pieces with a razor from time to time.

Borax-carminc may be prepared as follows (Grenacher):—Dissolve 1 gram. of carminc in 200 c.c. of a 1 per cent. solution of borax. Boil in a porcelain dish, and add a few drops of a

5 per cent. solution of acetic acid. The mixture is allowed to stand for a few hours and is then filtered. Thymol may be added for preservation.

Acid alcohol has the following composition :—

Hydrochloric acid	-	-	-	-	-	-	-	1	C.C.
Alcohol	-	-	-	-	-	-	-	70	C.C.
Water	-	-	-	-	-	-	-	30	C.C.

Methylated spirit may be used.

Picro-carmin and alum-carmin for sections, and borax-carmin for staining in bulk, are the most generally useful of the carmin stains.

2.—*Hæmatoxylin.*

Hæmatoxylin is one of the most valuable reagents, particularly for nuclei, but unfortunately the colour of sections stained with it fades in time. In this respect it is inferior to picro-carmin.

It is useful both for sections and for staining in bulk, and in the latter case, decolorisation with acid alcohol is not required, as the stain is not diffuse like borax-carmin. Kleinenberg's solution is perhaps generally the best. It should be diluted considerably before using, and the sections be allowed to remain in it for several hours. If rapid work is required, dilution with its own quantity of water will be sufficient. When stained sufficiently transfer to ordinary water.

When first removed from the hæmatoxylin, the sections have a purple colour, which changes in a few minutes to a brilliant violet blue, when they are laved in water.

From water they may be mounted in either Farrant's solution, or Canada balsam. The choice will depend considerably on the degree of transparency required. Most sections are better in the latter, but in the case of very thin ones, which are under, rather than over-stained, Farrant is perhaps to be preferred, especially when definition under the higher powers is aimed at.

If sections have become over-stained, it is often very advantageous to subject them to the action of weak acetic acid. A few drops of the ordinary acid, added to the water in which they have been placed, and gently stirred with a glass rod, in order to diffuse the acid thoroughly, is often sufficient to effect the purpose. The superfluous stain is thus rapidly dissolved out. The process requires a little care, however, to prevent the sections becoming completely decolorised. At the right time

they should be quickly whipped out with a needle, and placed in a large bowl of fresh water. Now wash very thoroughly in water, to remove every trace of the acid, otherwise it continues to act, even after the sections are mounted. It is better nevertheless to avoid the necessity for the use of acid, because it tends to dull the beautiful blue colour of the sections, and if carried too far, turns them red. The acid also seems to favour the fading of the hæmatoxylin, which always takes place in time, even though the sections have been thoroughly washed in water.

On the other hand, the addition of a few drops of ammonia, to the water in which the sections have been placed after staining, appears to accentuate the blue colour (Alexander).

A very beautiful effect may be produced by using first hæmatoxylin, and afterwards eosin, or picric acid. In the former case the sections, after being stained and washed in water, are placed in a .5 per cent. watery solution of eosin, which may be still further diluted if required. It stains the ground tissue of the sections a rosy red, while the nuclei remain picked out with the hæmatoxylin. Eosin specially stains the hæmoglobin of red blood corpuscles, certain granules in white corpuscles, and both striped and non-striped muscle. It stains with rapidity, only a few minutes being required. Mount in Farrant's solution, or balsam. In the latter case, it is well to clarify with clove oil in which eosin has been dissolved, the previous washing in alcohol being very apt to remove the stain.

Picric acid forms a charming adjunct to hæmatoxylin. If the sections are to be mounted in balsam, they are taken through alcohol in which picric acid has been dissolved. The ground structures thus become a bright picric yellow, the nuclei being stained with the hæmatoxylin. Their colour is, however, a purple red instead of blue, as the picric has somewhat the same effect as acetic acid on hæmatoxylin. Sections to be mounted in Farrant may be treated with picric acid on the slide, and mounted in the usual way. The acid must not be washed out with water.

Note.—Hæmatoxylin solutions require filtering from time to time, as a deposit readily occurs.

Hæmatoxylin (Kleinenberg).—This is perhaps the most

generally satisfactory of the many solutions of hæmatoxylin. It may be prepared as follows :—

1. Saturated solution of calcium chloride in 70 per cent. alcohol, with alum added to excess.

2. Saturated solution of alum in 70 per cent. alcohol.

Mix 1 with 2, in the proportion of 1 to 8.

To the mixture add a few drops of a solution, saturated, of hæmatoxylin in absolute alcohol.

It is a point of considerable importance that the solution should have the proper purple tint. It is very frequently at this stage red rather than purple, from the acidity of the solutions used. This may be corrected by the addition of a small quantity of a saturated solution of bicarbonate of soda in 70 per cent. alcohol. Experience readily enables the operator to gauge the amount required, which varies with the acidity. When the distinction between the real purple colour and the red has once been observed, it is readily recognized again.

Keep for a few days at least before using. Filter. Add thymol for preservation.

The following is also a useful formula for hæmatoxylin :—

1. Ammonia alum	-	-	-	-	-	3 grams.
Distilled water	-	-	-	-	-	100 c.c.
2. Hæmatoxylin	-	-	-	-	-	3 grams.
Absolute alcohol	-	-	-	-	-	16 c.c.

Mix and allow to stand for a few days. Filter.

A solution of logwood is sometimes used, though not so frequently as formerly.

Hæmatoxylin (Weigert).—Weigert's method of staining medullated nerve fibres with hæmatoxylin is of particular value in dealing with the central nervous system and nerve ganglia. The following is a serviceable method of employing the reagent.

Solution 1:—

A half saturated solution of cupric acetate, *i.e.*, a mixture of equal parts of a saturated solution of cupric acetate and water.

Solution 2 (Staining Solution):—

Hæmatoxylin	-	-	-	-	-	1 gram.
Absolute alcohol	-	-	-	-	-	10 c.c.
Distilled Water	-	-	-	-	-	90 c.c.

Mix the alcohol and hæmatoxylin in a mortar, and add the water. Heat the mixture for an hour or more on a sand bath, keeping the temperature just below boiling point. Allow

to cool, filter, and add 1 gram. of lithium carbonate. Allow the solution to stand for a day or two, and again filter. The colour should be purple, not red. If the latter, add more lithium carbonate.

Solution 3 (Clearing Solution):—

Potassium ferri-cyanide	-	-	-	-	2.5	grams.
Borax	-	-	-	-	2	grams.
Distilled water	-	-	-	-	100	c.c.

Dissolve and filter.

Sections of the brain and spinal cord should be first hardened in chromium salt, and in any case it is advisable to place them, after cutting and washing in water, in Müller's fluid for one or two hours. They are then again washed rapidly in water to remove the superfluous fluid, and transferred to Solution 1, in which they remain from three to twenty-four hours. Now wash in strong spirit and transfer to the staining reagent (Solution 2). Here they remain until the requisite depth of colour is attained. Roughly speaking from two to twelve hours are required. In the solution they become a deep brown or almost black. Now wash in water and immerse in Solution 3. This will extract a good deal of the stain, leaving the grey matter yellowish or reddish brown, but the medullary sheath of the nerve fibres violet or dark blue. The clearing fluid may require renewing during the process, the time required for which will depend on the thickness of the sections and the extent to which they have been stained in Solution 2, and may vary from a quarter-of-an-hour to several hours. Experience, however, readily decides. They are then washed in water, and either mounted in balsam after dehydrating and clearing as usual, or preserved in spirit. The colour in the latter case is very liable to fade.

One of the chief difficulties encountered in this method, especially when a large number are required, is the tendency of the sections to break up, under the constant handling they are subjected to in transference from one solution to another, washing, etc. To overcome this, see in the first place that the piece of tissue to be cut is satisfactorily hardened. Any brittleness is very fatal (*see* "Central Nervous System"). Sections of spinal ganglia, however, usually present no difficulty of this sort.

3.—*Eosin*.

A 5 per cent. watery solution may be made and diluted as required. It is used in strengths varying from 1 in 100 to 1 in 2000. It is a diffuse stain, and the stronger solutions act in a few minutes. It is frequently used in contrast with others, especially nuclear stains, such as hæmatoxylin — the hæmatoxylin colouring the nuclei blue or violet, and the eosin the perinuclear portion of the cells a rosy pink. It is particularly useful for striped muscle fibres and blood corpuscles. When the solution used is weak, sections may be left in it for some hours. They may be mounted in either Farrant's solution or balsam. Eosin is soluble in alcohol; it is therefore well to use clove oil in which a little eosin has been dissolved as a clarifying reagent.

B.—ANILINE STAINS.

Aniline stains are exceedingly valuable for special purposes, which will be more fully referred to in the text. They are largely used for cover-glass preparations in bacteriology, and also for those which are made for ordinary histological purposes. They are not adapted for treating tissues in bulk, on account of the readiness with which the stain is removed by water, alcohol, and other reagents. Specimens should be mounted in balsam in preference to Farrant, as the latter removes the stain. Cover-glass preparations may be mounted directly in balsam without previous clarification. Sections should be clarified with cedar oil or xylol in preference to clove oil, which in many cases causes the stain to dissolve out. The process of dehydration before clearing should not be unnecessarily prolonged, in order to wash out as little of the aniline as possible.

The following are some of the more commonly used aniline stains. Usually a weak solution, .5 per cent., is sufficient to stain the tissues in a few minutes. Watery solutions are used, to which a certain amount of alcohol is usually added.

Methyl-blue.—Amongst other applications methyl-blue in strengths of .1 to 1 per cent. is especially useful for cover-glass preparations of blood, and of the grey matter of the spinal cord. It is also a striking stain for sections of the cardiac end of the stomach. It is a nuclear stain, and acts very rapidly.

Spiller's Purple.—Useful for the filaments of fibrin in coagulated blood. Use a 1 to 2 per cent. solution.

Iodine-green.—This is a good nuclear and contrast stain. Use a 5 per cent. watery solution.

Methyl-green.—Methyl-green is a nuclear stain of great service in double-staining, especially for mucous glands and the hyaline matrix of cartilage. To 100 c.c. of a 1 per cent. watery solution add 10 c.c. of alcohol.

Magenta (sulphate or acetate of rosaniline).—Magenta has the serious disadvantage of not being permanent. Useful for nuclei generally, for staining blood corpuscles and elastic fibres.

The following solution may be used for blood corpuscles :—

Magenta	-	-	-	-	-	-	1 gram.
Distilled water	-	-	-	-	-	-	15 c.c.
Rectified spirit	-	-	-	-	-	-	5 c.c.
Glycerine	-	-	-	-	-	-	20 c.c.

For other purposes take a weaker solution :—

Magenta	-	-	-	-	-	-	1 gram.
Distilled water	-	-	-	-	-	-	191 c.c.
Rectified spirit	-	-	-	-	-	-	9 c.c.

Saffranin.—For the study of mitosis this is especially useful. The tissues should be first hardened in Flemming's fluid. Dissolve 1 gram. of saffranin in a mixture of alcohol and water ; 100 c.c. absolute alcohol to 200 c.c. distilled water. When the sections have been cut in paraffin, fix them in the ordinary manner on a slide. Remove the paraffin with turpentine, and the turpentine with absolute alcohol. Apply the stain in considerable quantity, and leave the slide in a moist chamber for some hours. Remove the superfluous stain, and apply acid alcohol for a few moments. This removes the colour from all but the chromatic threads of the nuclei ; but as its action is very rapid and may easily be over-done, it is perhaps safer, till the process becomes familiar, to use ordinary alcohol for decolorisation. This will produce the same effect, but much more slowly. After dehydration the sections are cleared and mounted in balsam.

Bismarck Brown.—Is useful for staining the cortex of the cerebrum. Use a 1 per cent. solution. It colours the nuclei of the cells dark, and the protoplasm a lighter, brown.

Aniline Blue-black.—Particularly useful for sections of the brain and spinal cord. It has a special affinity for the nerve cells, and is not so readily washed out as in the case of other aniline stains. It requires at least some hours to act, and sec-

tions may often be left in weak solutions for two or three days. They are then dehydrated, cleared, and mounted in balsam.

C.—METALLIC STAINS.

Silver Nitrate.—Silver nitrate forms a combination with interstitial cement substance which becomes brown or black on exposure to light, from reduction of the silver salt. It is thus used for demonstrating the outlines of epithelial cells; especially in the case of simple squamous epithelium, and the cell spaces of connective tissue. In the latter case, what is termed a negative image is produced, the whole ground substance of the tissue being coloured brown, while the spaces in which the cells lie are neglected. This staining of connective tissue is therefore nearly the exact counterpart of that obtained with gold chloride (*see infra*), which gives a "positive image," the cells lying in the spaces being stained deeply, while the surrounding matrix is only faintly so.

As it is exceedingly important that the student should succeed with this method, the following three examples will be dealt with in some detail.

(a) *Omentum.*—To stain the omentum with silver nitrate a $\frac{1}{4}$ per cent. solution of the reagent may be employed.

Cut out a piece of the omentum from a cat or rabbit recently killed. Lave rapidly in distilled water for a few seconds, to remove as much as possible of the chlorides, which precipitate silver, without, however, prolonging the immersion sufficiently to injure the delicate epithelial cells covering the membrane. Transfer to a glass or porcelain vessel containing the silver solution, noting that the omentum floats freely, so that it is fully exposed to the action of the fluid. After ten to twenty minutes, transfer to a mixture of 1 part of spirit to 3 of water in a porcelain dish. See that the membrane is spread out as much as possible. It is desirable to use a white porcelain vessel, in order that the light may strike upwards to the under surface. If the vessel be glass, almost the same effect is produced by placing it upon a sheet of white paper. Cover with a glass plate and expose to good daylight until it becomes brown on its upper surface; it may then be turned, and the previous under surface subjected to light from above. The colour should be something between that of burnt sienna and vandyke brown, but the only

satisfactory method of determining when the process is complete is to snip off a piece of the tissue, and examine it under the microscope. The cells covering the fibrous trabeculæ should have their edges revealed as dark lines. If this reaction has not occurred, or only imperfectly, the exposure to light must be continued.

This process usually occupies from a few hours to a day or two. Staining either with silver nitrate or gold chloride, however, is always more or less uncertain in its results, and the time necessary for its completion varies considerably. It has also to be noted that the process of reduction continues after the tissue has been put away in spirit for preservation, and after specimens are mounted. Thus, even when success is not at first fully attained, considerable improvement may take place later.

Subsequently the tissue may, if desired, be further treated with some nuclear stain, such as picro-carmin or hæmatoxylin, and then mounted in Farrant's solution or Canada balsam.

(b) *Tendons of rat's tail*.—The fine tendons of a rat's tail may be advantageously stained with AgNO_3 to show (1) the outlines of the epithelial cells covering them; (2) the cell spaces in the tissue itself. Kill a rat, hold the tail after removal with both hands, and with a sharp turn fracture the bony ligamentous element in it at the point. Pull the tail into two parts. The skin gives way at the site of fracture, but the tendons rupture at some more distant point, and hang as a leash to one part of the tail. Use this part as a handle with which to manipulate the tendons. Immerse them in a shallow porcelain dish containing distilled water, and with a camel's hair brush "pencil" them rather roughly. The distilled water removes the common salt, and the brushing a good deal of the epithelium, so that the fibrous tissue beneath it is exposed.

Now clip away the tendons from the piece of tail and place them in $\frac{1}{4}$ per cent. nitrate of silver solution in which they remain ten to twenty minutes. At the end of that time expose them to light, as in the case of the omentum. These tendons require, however, to be clarified with clove oil and mounted in Canada balsam. They are of course very much thicker than sections, and as much transparency as possible is to be aimed at. It is well to perform the processes of dehydration and clarification, not upon the slide, but in watch-glasses, and allow plenty of time for thorough saturation in each case.

Under the microscope, here and there where the epithelium has been left, the outlines of its cells will be seen, and where it has been brushed away the cell spaces between the fibres of connective tissue should be looked for (*see* "Tendon").

The central tendon of the Diaphragm is dealt with in much the same way. It is cut out of the body with as little muscle round it as possible, placed in distilled water, and one of its surfaces at least may be thoroughly pencilled with a camel's hair brush, to remove the epithelium covering it, if it is desired to show the cell spaces beneath. It is then transferred to silver nitrate, and afterwards to spirit and water in which it is exposed to light. Pieces of this tissue also require, on account of their thickness, to be clarified in clove oil very thoroughly.

(c) *Cell spaces in Cornea*.—Remove the eye of a frog. With a scalpel scrape away the anterior epithelium, and rub the denuded surface with solid silver nitrate for a few minutes. Lave in distilled water; excise the cornea; mount in Far-rant's solution. Exposure to light in this case takes place after mounting. Clove oil is not required because of the natural transparency of the cornea. The ground substance of the cornea is stained brown, the cell spaces being unaffected.

Silver nitrate has other applications, but these will be dealt with as they occur in the text. The above four are merely given as descriptions of the method employed. It is well to keep a stock solution of 1 per cent. and dilute it as required. It should be kept in bottle, covered with brown paper. For most purposes $\frac{1}{4}$ per cent. is the most suitable strength in which to use it. If a precipitate forms in the stock solution it should be filtered before being used. The necessity for washing tissues in distilled water should be noted; if the chlorides are not thus removed a milkiness results, when the pieces are transferred to the solution, due to the precipitation of chloride of silver (AgCl).

Silver nitrate also acts as a hardening reagent.

Gold chloride.—Gold chloride is particularly serviceable for nerve plexuses, nerve terminations, and the cells of connective tissues.

There are several modifications of the process described. One of the simplest is the following:—Make a 1 per cent. solution of gold chloride. Take 4 parts of this and mix with 1 part of formic acid. Boil the mixture once or twice, and then

allow to cool. Place pieces of the tissue (cornea, tendon or muscular wall of intestine for Auerbach's plexus) in the fluid for fifteen minutes or longer. The tissue swells up and becomes yellow and transparent. Wash in water and transfer to a solution of formic acid (20 per cent.), and keep in darkness for twelve to twenty-four hours. As reduction takes place, the tissue becomes purple. Inasmuch as the tissues are rendered transparent by the method itself, they do not necessarily require mounting in balsam. Pieces of the tendons from the rat's tail may be placed on a slide (after washing away the formic acid), and mounted in Farrant's solution. A little pressure on the cover glass with a needle will usually dissociate the tendon bundles to a sufficient extent to demonstrate the tendon cells. Gold chloride leaves the nuclei of the cells unstained. The muscular wall of the intestine for Auerbach's or Meissner's plexus is better mounted in balsam, and dehydration and clarification should be carried out in watch-glasses. Like silver nitrate, gold chloride is very uncertain in its results, and the student must be prepared for a good many failures.

Acetic Acid Method.—Small pieces of the tissue are placed in $\frac{1}{2}$ per cent. solution of gold chloride, and kept in the dark for half an hour. They are then transferred to acidulated distilled water, and exposed to daylight for twenty-four to forty-eight hours.

Lemon-juice Method (Ranvier).—Place small pieces of tissue in fresh lemon-juice for five to fifteen minutes, wash in distilled water, and place in gold chloride 1 per cent. for fifteen minutes to one hour. Transfer to formic acid 20 per cent., and keep in the dark for twenty-four hours.

Rapid staining with Gold Chloride.—Place the pieces of tissue in 1 per cent solution of gold chloride, for half to one hour, transfer to strong solution of tartaric acid, and heat to 50° C. Reduction takes place in a few minutes.

Osmic Acid.—Osmic acid is employed as a reagent for blackening the fat of fat cells, the medullary sheath of nerves, etc. It is also a hardening reagent, and gives definition to the outlines of epithelial cells. It darkens the tissues generally. A stock solution of 1 per cent strength should be kept in a bottle preserved from the light. It is used in solutions, $\frac{1}{10}$ to 1 per cent.; $\frac{1}{4}$ per cent. is very commonly used for staining purposes.

D.—COMPOUND STAINS.

Cover-glass preparations, or sections, may be stained first with one reagent and then with another, and it may be with a third ; or, they may be placed at once into a mixture of two or more stains, each of which specially affects some part of the specimen.

Picro-carmin.—(*see ante.*)

Hæmatoxylin and Eosin.—The sections are placed first in a weak solution of hæmatoxylin, washed, and transferred to eosin—I in 1000 solution. They are then rapidly washed and mounted in Farrant or balsam. The nuclei become blue or violet, and the perinuclear protoplasm rose pink.

Hæmatoxylin and Picric acid.—First stain in hæmatoxylin, and then subject for a short time to picric acid (saturated solution). If the sections are to be mounted in balsam, the alcohol for dehydration should contain picric acid. The ground structures will show yellow, and the nuclei purple—inclining to red, according to the extent of the action of the acid.

Hæmatoxylin and Aniline blue.—This is a useful method for a “mixed” salivary gland. The sections are stained first in hæmatoxylin, and then for a short time in methyl-blue. The serous cells are coloured by the hæmatoxylin, while the mucous are picked out by the aniline.

Ehrlich-Biondi Stain.

Saturated watery solutions of	{	Orange	100 c.c.
		Acid fuchsin	20 c.c.
		Methyl-green	50 c.c.

The mixture is diluted with 100 times its volume of water before use, and on the addition of acetic acid becomes bright red. It is said to act best on tissues which have been hardened in corrosive sublimate. The sections are placed in the solution from twelve to twenty-four hours, and then dehydrated and mounted in balsam. It is, however, uncertain in action, and sometimes even half-an-hour is sufficient. It is especially useful for red and white blood corpuscles, and for the mitotic figures in dividing cells.

In addition to those mentioned above, there are many other combinations, *e.g.*, Carmine and Methyl-blue, Eosin and Methyl-green, Picro-carmin and Methyl-green, etc.

CLEARING REAGENTS.

Clearing reagents are used for those sections which are to be mounted in Canada balsam, xylol balsam, or dammar lac.

Clove oil is most generally adopted ; but it cannot be used for sections cut in celloidin, which it dissolves, nor for sections stained in aniline, for a similar reason.

Turpentine acts as a clearing agent, at the same time that it removes paraffin from sections cut in it.

Xylol is very useful, especially in the case of sections cut in celloidin, or stained with aniline dyes. The balsam should be dissolved in xylol rather than in benzol.

Origanum oil, cedar oil, bergamot oil, are also used.

MOUNTING FLUIDS.

There are two chief classes of mounting fluids : (1,) Those containing Glycerine, which are miscible with water ; (2,) Those containing Resin, which are not. Sections may be mounted directly from water in any of the first class ; they must be dehydrated and cleared before being mounted in the second.

GLYCERINE MOUNTING FLUIDS.

Pure glycerine.—Pure glycerine may be used for sections stained in picro-carmin, but is not so generally useful as Farrant's solution. It does not "harden" at the edge of the cover-glass, as Farrant does, and hence gives rise to some trouble when the specimen has to be ringed. It renders tissues, however, more transparent, which is an advantage in some cases.

Farrant's solution.—This is much the most generally useful of the glycerine series. Equal parts of water, glycerine, and arsenious acid (watery solution : saturated) are taken ; gum arabic is added and allowed to dissolve gradually, usually occupying a few days at least in the process. The mixture must contain sufficient gum to give the consistence of thick syrup. Filter through several thicknesses of muslin before using.

Glycerine jelly.—Glycerine jelly is not employed as an alternative to either of the above, but for special purposes. It is exceedingly useful for the preservation of cells (already

hardened) in an isolated condition, or for urinary crystals, which have been diffused through it, while in a melted condition (*see ante* page 4). When thus used the tube containing it is placed in hot water, and when the jelly is melted a glass rod is dipped in it, and a drop of the fluid transferred to the slide and covered. The cover-glass should be applied quickly, so that the jelly has no time to set. Should this have occurred, and the fluid in consequence not spread between the cover and the slide, hold the latter a moment or two over the flame of a Bunsen burner which will remelt it, and rectify the error. Any superfluous fluid at the edge of the cover may be readily removed with a piece of rag, or, after it has set, with a penknife.

RESIN MOUNTING FLUIDS.

Benzol balsam.—This is a solution of Canada balsam in benzol, and is the form in which it is most usually employed. The benzol evaporates on exposure to the air, and the balsam gradually thickens. The addition of a little more of the solvent will readily thin it again.

The balsam should be dried before being dissolved.

Xylol balsam.—In this case xylol is used as a solvent. Its special use for the mounting of sections, cleared with xylol, has already been indicated. By some this solvent is preferred to benzol for general use.

INJECTION OF BLOOD-VESSELS.

Blood-vessels may be injected with : (1.) Transparent coloured gelatine mass ; (2.) Opaque mass ; (3.) Watery solutions, *e.g.*, of prussian blue ; (4.) Solutions of staining reagents, *e.g.*, nitrate of silver. The first of these will be considered here, the others are referred to in the text, together with the injection of gland tubes and of lymphatics (interstitial injection). (*See Index.*)

Gelatine injections.—The principle of this method is the filling of the vessels with a melted gelatine mass, holding, diffused through it, some coloured substance, such as carmine, or prussian blue. When the gelatine solidifies on cooling, we have a solid coloured, transparent injection marking out the arteries, veins, and capillaries of the organ injected.

The following are the injection masses usually employed :—

Carter's Carmine Gelatine Mass.

Carmine	-	-	-	-	-	-	3 grams.
Strong ammonia	-	-	-	-	-	-	6 c.c.
Glacial acetic acid	-	-	-	-	-	-	6 c.c.
French gelatine	-	-	-	-	-	-	7 grams.
Water	-	-	-	-	-	-	80 c.c.

The ammoniacal solution of the carmine and the gelatine solution are prepared separately, 50 c.c. of water being used for the latter, and 30 c.c. for the former. Add glacial acetic acid drop by drop to the carmine solution, ceasing as soon as it becomes crimson. It is very important to stop at the point of neutralisation. Keep the fluid in motion by means of a glass rod while the acid is being added.

The melted gelatine and the carmine solution are now mixed, and the mixture filtered through flannel. The filtration should take place in a funnel kept at a sufficiently high temperature by means of a water jacket. If this precaution is not taken the process is soon arrested by the solidifying of the mass. After filtration and subsequent solidification, add sufficient spirit to cover the surface, for preservation. It may be again stated that it is essential that the mass should be neutral; if too much acetic acid is added to the carmine the latter is precipitated, and may be detected on the sides of the glass vessel containing it, if slightly tilted and held up to the light. Again, if the carmine solution is distinctly alkaline, the colouring matter is apt to diffuse from the vessel into the tissues, and play the part of a stain.

Blue Injection Mass.

Solution 1	{	Soluble prussian blue	-	-	-	4 grams.
		Water	-	-	-	300 c.c.
Solution 2	{	Gelatine	-	-	-	33 grams.
		Water	-	-	-	200 c.c.

Add 1 to 2; filter as before. When cool cover with a layer of spirit.

The fluid to be injected may be thrown into the vessels, either by means of a hand syringe, or by what is termed the continuous air pressure method. As a general rule, the latter has very much to recommend it, because any variation in the pressure is easily avoided. With a little practice however, a syringe may be used with tolerable safety. The principal danger is the liability of the blood-vessels to rupture from sudden, unintentional increase of pressure.

To inject the blood-vessels of an animal with a hand syringe.—Place the animal deeply under chloroform, expose the contents

of the chest cavity, and with the scissors cut through the wall of the right auricle or ventricle, soaking up the blood with a moist sponge. Now cut off the apex of the left ventricle, and through the aperture insert the nozzle of a glass cannula into the aorta; pass a thread with an aneurism needle round the base of the vessel above the heart, and finally ligature the cannula in position. The animal must now be placed in a bath containing hot water, the temperature of which should be a few degrees at the least above that of the normal temperature of the body. Warm the syringe by repeatedly filling it with hot water, and emptying it again; finally, fill it with the melted gelatine mass, and connect it with the cannula by means of a piece of india-rubber tubing. It is well to tie the tubing firmly by means of a piece of twine to both the cannula and the syringe, as otherwise it is liable to slip when pressure is exerted. Now commence steady, even pressure with the piston of the syringe, combining the pushing with a rotatory screw movement. Progress may be judged by watching the colour of the gums and mucous membrane of the mouth generally; as it proceeds they become coloured by the injection mass. When the process is considered complete, the animal should be placed in running water so as to cool it as quickly as possible; the organs are then removed, cut into pieces, and hardened. More than one syringe full of the gelatine mass may be required.

To inject the blood-vessels of an animal by means of continuous air pressure.—The fluid in this case is contained in a Wolff's bottle, which is kept in the hot water with the animal. The bottle is connected on the one hand with the cannula in the aorta, and, on the other, with a larger bottle containing air, and the latter, by a second tube, with a water tap! A connection is also made by a third tube with a manometer. Thus, when the tap is turned, water flows into the bottle containing air, which thus becomes compressed. As the water rises at the bottom of the bottle it forces the air (1,) into the tube leading to the manometer *which registers the pressure*, and (2,) into the tube leading to the Wolff's bottle. Here the compressed air meets the surface of the melted injection mass, and forces it up into the tube (which reaches nearly to the bottom of the bottle) connected with the cannula in the aorta.

This method is far the more delicate of the two. It is not however, unfortunately, so simple. The main points to be

attended to are the following : (1,) See that the whole system is air-tight, any leakage being fatal ; (2,) See also that all the connections between the glass tubes leading into the bottles and the india-rubber tubing connecting the bottles with each other, and with the tap and the cannula, are securely fastened, else the connections may slip under pressure ; (3,) Prevent the gelatine mass setting in the tube between the Wolff's bottle and the cannula by keeping it sufficiently warm.

When an organ by itself is to be injected it may be removed from the body, and treated by either of the above methods. It is usually possible to get a satisfactory injection with a hand syringe. The cannula is fixed in the main artery.

APPENDIX TO CHAPTERS I. AND II.

HISTOLOGICAL REQUISITES.

The Microscope.—As the student now has usually had some experience of microscopical work in biology, before commencing histology, it is scarcely necessary to do more than mention the more important points to be attended to in selecting a microscope. The stand should be firm, that is to say, the instrument should not readily over-balance if it is tilted slightly in any direction. The stem should be jointed between the stage and the foot, so that the tube may be inclined towards the observer at any angle desired. If there is no joint and the stem is rigid, the microscope can only be used in the upright position, frequently a source of great inconvenience. The tube should be a single one, not a binocular as it is termed, and it should not be too long. It is advisable to limit one's self to one eye-piece, a weak one, and to vary the magnifying power by means of the lenses. Of these, two are sufficient for all ordinary purposes, and should magnify about 50 and 350 respectively. If expense is no object, an additional lens magnifying 600 may be added. The lenses should be examined with great care : (1) as to their definition ; (2) as to the flatness of the field.

A *nose-piece* is an essential for comfortable work. If the lenses have to be constantly screwed on and off the tube of the microscope, much time and energy are wasted. With a nose-piece, a touch of the finger enables the operator to change from one power to another instantly. The upper half of the nose-piece is screwed to the tube ; the lower carries the lenses and rotates upon the upper. By rotation, first one and then the other lens can be brought into line with the tube as required. Some nose-pieces are adapted to bear three lenses. The microscope must have two *adjustments*, a fine and a coarse one. The latter is made by sliding the tube up and down in its socket, by hand or by a rack and pinion ; the former is worked by rotation of a milled head, placed at the top of the stem of the instrument. This head is connected with a screw, which acts upon a spring, and thereby raises or lowers the tube. It is particularly important that the fine adjustment should work smoothly and evenly, as upon the perfection of this part of the instrument depends the accuracy of focussing attainable.

The choice of a microscope is largely a question of expense and individual taste. There are many admirable instruments now in the market, which the student should see before making his selection. It would be almost invidious to recommend any particular maker. The microscopes of Zeiss, Hartnack, Pillisher, Beck, Swift, Leitz, and others are all excellent.

If economy is a desideratum, Beck's "Star" microscope can hardly be surpassed. The cost is only about £3, the lenses are good, the microscope stands firmly, it is jointed, the fine adjustment is simple and not liable to get out of order.

It frequently happens that the student already possesses an inherited microscope. Unless really satisfactory, it is far better to dispose of it and get a modern one, than attempt to use it. The length of the tube, the inferior quality of the lenses, the complexity of the stage, and the imperfections of the fine adjustment, render many of these instruments more trouble than they are worth. The mechanical stage substituting an arrangement of levers and milled heads for the delicate intuitive movements of the fingers is very objectionable.

For working with higher powers, it is advisable to have a substage *condenser*, which increases the illumination by concentrating the light from the mirror. Abbé's condenser is very excellent, but cheaper ones may be obtained.

In addition to a condenser in using the highest powers, it is of advantage to have a fluid between the lens and the cover-glass of a refractive index more nearly approaching that of the glass than air. For this purpose oils of various kinds may be used, such as a mixture of fennel and ricinus oils, cedar-wood oil, etc. : a small drop is placed with a glass rod upon the cover-glass, and the lens carefully brought in contact with it. A high power lens, say $\frac{1}{12}$ inch, with which oil is to be used, is termed an *oil immersion* lens. There are also water-immersion lenses, with which a drop of water is used instead of oil. These immersion lenses have more resolving power, and a larger angle of aperture than the dry lenses, in using which, air alone intervenes between the objective and the cover-glass.

A Camera lucida is an important adjunct to the microscope, for the student of histology. Roughly speaking, it consists of an arrangement of prisms placed above the ocular. On looking through the camera, the operator sees the field of the microscope reflected upon a piece of paper, placed upon the table or at the level of the stage. It is very easy with a little practice, to follow the outlines of the specimen thus reflected, and so get a perfectly accurate drawing. The instruments manufactured by Nachet and Verick of Paris, are both easy to work with and comparatively inexpensive.

A Micrometer is very useful in microscopical work. Micrometers consist of scales drawn on glass, and of these there are two kinds :—

1.—The stage micrometer, consisting of a glass slide with, say, two scales upon it of $\frac{1}{100}$ and $\frac{1}{1000}$ millimetre respectively.

2.—The eye-piece micrometer, which is a circular piece of glass with a scale upon it, placed between the upper and lower glasses of the ocular, *i.e.*, between the eye-glass and the field-glass.

To estimate the size of an object such as a crystal, or a blood corpuscle, first of all focus it. Place a piece of paper on the right side of the microscope, and look through the tube with the left eye, keeping both eyes open. The object to be measured, appears on the sheet of paper, and an outline can be rapidly made of it with a pencil. Now remove the specimen and substitute the stage micrometer, marking off its scale upon the paper in the same way; it will first require focussing. It is then simple to calculate the

size of the object. If it covers $\frac{2}{3}$ of the space between two lines of the $\frac{1}{100}$ millimetre scale, its size is $\frac{2}{3} \times \frac{1}{100}$ of a millimetre, *i.e.*, $\frac{2}{300} = \frac{1}{150}$ of a millimetre.

The eye-piece Micrometer is used as follows: Insert the eye-piece containing it in the tube, and place the stage micrometer in position. Focus the scale on the stage micrometer, and contrast with it the scale in the eye-piece. If 2 divisions in the eye-piece = 1 on the stage (of the $\frac{1}{100}$ scale):

then 2 divisions = $\frac{1}{100}$ of a millimetre.

1 division = $\frac{1}{200}$ " "

As soon as the value of the divisions of the eye-piece scale, with the particular lens used, is thus determined, the slide, with the object to be measured, is substituted for the stage micrometer, and it is ascertained how many divisions of the eye-piece scale are required to cover it. If 10, then its diameter is $10 \times \frac{1}{200}$ or $\frac{1}{20}$ of a millimetre.

It is easy with the stage micrometer to ascertain the magnifying power of different combinations of eye-pieces and objectives, and with the draw-tube of the microscope in and out. The scale is focussed and marked off on paper, at the side of the microscope. If the distance between the lines on the paper is one millimetre, then if it is the $\frac{1}{100}$ m. scale which is being examined, the magnification is 100; if the $\frac{1}{1000}$ m. scale is being examined, the magnification is 1000.

The unit of measurement in histology is $\frac{1}{1000}$ part of a millimetre, frequently spoken of as one micron, and expressed by the letter μ .

Slides and cover-glasses.—The most useful size of slide is 3 in. \times 1 in., but larger ones 3 in. \times 1½ in. are from time to time required.

Cover-glasses are either square or round. The round should always be used for specimens which have to be ringed, *i.e.*, those mounted in Farrant, glycerine, or glycerine jelly. They are of sizes to correspond with the slides: $\frac{3}{8}$ in. and $\frac{3}{4}$ in. are useful sizes for the small slides, and 1 in. or 1¼ in. for the large. They are manufactured of different thicknesses. No. 3 will do for ordinary work. For working with really high powers, use Nos. 1 and 2. Cover-glasses require careful cleaning before using.

Boxes for slides.—These are of various kinds, and the selection of one is greatly a matter of taste. It should be capable of holding at least 120 slides, and it is better if it will hold 180 or 200.

A Section lifter.—This is made from a rod of copper wire, with one end beaten out and bent at an angle.

Needles in handles.—These are constantly used for floating sections upon the slides in mounting, and also for teasing tissues. It is well to have four of them to start with.

Camel-hair brushes.—One or two small brushes are often useful, but, generally speaking, the needle is much to be preferred in handling sections.

Biological instruments.—These should include a fine pair of scissors, a sharp scalpel, and a razor ground flat on one side.

Watch-glasses.—A few watch-glasses 3 inches in diameter are needed for staining, clearing, etc.

Labels.—A supply of labels, 1 inch square for the small slides, and 1½ in. \times ¾ in. for the large, is very necessary.

Reagents.—These are supplied by the department. The student requires to have the following on the table before him : Picro-carmin, hæmatoxylin, absolute alcohol, oil of cloves, Canada balsam, acetic acid (dilute 1 per cent). normal saline, and Farrant's solution.

Drawing materials.—It is very important that the student should make drawings of his specimens. This necessitates his thoroughly understanding the structure he is examining. A drawing made without knowledge is of little value. Drawings should be in colours by preference, rather than mere pencil sketches. It fixes the action of the colour reagents upon each tissue very firmly in the mind, the picture is much more intelligible for future reference, and the effect much more pleasing to the eye. With the pencil, first put in the general outlines, then the outlines of the cells and fibres, and the nuclei. Then put in the ground work of the nuclei and the perinuclear protoplasm. This is easily done by dotting with the pencil so as to give a granular appearance, that of the nucleus being made a little the heavier. After this, the ground colours, light pink, or blue, or yellow, as the case may be, should be laid on with the paint brush ; and when they have dried, the deeper tints of the cell protoplasm, nuclei, and fibres should be put in.

The paper used should be thick, and not glazed. It may even be a little rough with advantage, but not as rough as cartridge paper which would interfere with the accurate working in of the finer details. It should be slightly tinted, a faint yellow or cream being preferable, but the yellow must not be deep enough to interfere with any yellow in the drawing. A dead or a blue white gives a hard appearance to the figures.

To make really satisfactory drawings, it is well to have two sketch books, one for taking rough drawings in class, and another for making the finished ones at home. It is almost impossible to make a satisfactory set of drawings during the meetings of the class. A certain amount of reading always requires to be done, and a good deal of quiet study of the specimen, before making a drawing, and the opportunity for this only occurs when the student is at leisure. It cannot be too emphatically stated, that in the study of histology, no time can be better spent than in drawing the preparations. By the time all the specimens have been carefully drawn, the student will find that he has a genuine knowledge of his subject, which it is almost impossible to obtain in any other way.

The ordinary materials for water-colour painting will do quite well. A small box of moist colours, an HHH pencil, a few brushes of different sizes, and indiarubber, being all that are required. The pencil should be a thoroughly good one, and as already stated, hard. With a soft pencil the lines are far too heavy.

CHAPTER III.

*THE SIMPLE TISSUES:
THE ANIMAL CELL. BLOOD AND LYMPH.***THE ANIMAL CELL.**

AN animal cell consists essentially of two parts—the nucleus and perinuclear protoplasm. Starting from this, the typical condition of an active cell (represented in the body by the white blood corpuscle), it may in the process of development acquire a cell wall, or, as it is also termed, a periplast. It then ceases to be capable of movement from place to place, and becomes fixed in position. The periplast may vary very much both in nature and degree. It may amount merely to an increase and condensation of the peripheral part of the protoplasm, forming either a complete wall or an incomplete one, perforated by pores which readily admit of a rapid interchange of fluid between the interior and exterior of the cell; or new material may be produced to such an extent as to result in the formation of a fibrillated ground-work between the cells, as in the case of cartilage and bone. In these cases the fibrillated periplast may almost be said to be “secreted” by the cells, but the exact nature of the process we do not understand.

Again, a cell in its development to serve certain functions, may undergo changes and lose its nucleus and perinuclear protoplasm, or these may become converted to some simpler substance. This is the case with the cells of the stratum corneum of the skin, which have virtually ceased to have any function but a mechanical one, their periplast, or cell wall—the most stable and inactive portion—enclosing a homogeneous substance keratin, into which its contents have been converted. Such a cell may be considered to have lived its life and died, the soft parts or endoderm having degenerated, and only the skeleton or ectoderm persisting unchanged.

In the white blood corpuscle (the amœba of the blood), we have the essentials of a typical active animal cell; a nucleated protoplast, or mass of protoplasm furnished with a nucleus. From this we may first step to the cells of the epithelia, which have acquired a cell wall, the completeness of which varies in different cases. After this we reach the stage of cells placed interstitially in a matrix, the fibrillated part of which has been produced under their influence.

The nature of the typical active cell may be studied in the white blood corpuscle.

Protoplasm.—The protoplasm of the cell consists of two parts, the spongioplasm or network, and a fluid substance, occupying its meshes, the hyaloplasm or enchylema. When amœboid changes take place, resulting in the thrusting out of pseudopodia, the clear homogeneous hyaloplasm first flows out from the meshes of the spongioplasm which follows it more gradually. The hyaloplasm may perhaps therefore be regarded as the active contractile part of the corpuscle. If the cell be stimulated in any way while executing amœboid movements, the pseudopodia of hyaloplasm flow back into the spongioplasm, the processes of the latter are themselves retracted, and the spheroidal condition of the corpuscle is resumed. This condition is retained as long as the corpuscles remain in the blood stream, probably from the mechanical stimulation they receive from impact against other cells and the blood-vessel wall. It is only after the blood has been drawn from the vessel and allowed to remain quiescent for a while that the amœboid movements begin. They also take place in the body, when the cells escape from the blood-vessels into the tissues, and become leucocytes or wandering cells. It is in virtue of this amœboid power that they escape from the vessel, a pseudopodium being thrust between adjacent epithelial cells, and the rest of the hyaloplasm and the spongioplasm flowing in the same direction till the whole has passed through. This passage of the amœboid cells takes place particularly during the stage of stasis in inflammation, when the current of the blood is very much slowed.

The spherical condition of the cell is perhaps comparable to the contracted state of a muscular fibre, and the pseudopodic state to that of extension. By this process of sending out pseudopodia or a pseudopodium in a certain direction, followed

by the body of the cell floating after it, the corpuscles are enabled to travel from one situation to another.

The Nucleus of the Cell.—The nucleus is usually a round or oval body, placed towards its centre. It stains more deeply than the perinuclear protoplasm and, like it, consists of two parts; a network of what has been termed chromoplasm, containing in its meshes a more or less fluid substance, the nuclear matrix. The whole is enclosed by a distinct nuclear wall or membrane.

At certain points in the nucleus one or two nucleoli or small rounded bodies may be seen, which have been variously regarded as collections of material at the nodes of the network, and as distinct, functionally and chemically, from the rest of the nucleus.

But though the chromoplasm is usually found arranged in a network connected with the wall of the nucleus, this is not invariably the case. When the process of division or karyokinesis is about to commence, it is found in the form of a convoluted thread.

Nature of the Chromoplasm.—It is not structureless. Its nature was first indicated in the salivary gland cells of *Chironomus*. It consists of a homogeneous ground substance, which is unstained by reagents, termed achromatin, and a substance imbedded in it in the form of granules, which stains readily and which has been called in consequence, chromatin. It is to the staining of these particles of chromatin that the deeper colour of the nucleus is due.

Function of the Nucleus.—The nucleus of a cell appears to have an essential part to play both in its nutrition and in the formation of a cell wall. It is stated that a piece of protoplasm separated from the rest of the cell will continue to live and even ingest food particles, but that it will not digest them, nor form a periplast. Whatever the truth of these statements may be, its only function, of which we may consider we have any certain knowledge, is connected with the reproduction, by division, of the cell.

Division of the Cell.—This may be either direct or indirect. In direct fission the division of the cell protoplasm is preceded by that of the nucleus, a constriction appearing about the centre of the latter, and proceeding until it is separated into two parts or daughter nuclei. The protoplasm of the cell then divides, and the two parts remain around their

FIG. 1.

DIAGRAMMATIC REPRESENTATION OF STAGES OF KARYOKINESIS.

- a.*—Cell with resting nucleus.
- b.*—Convolution stage.
- c.*—Wreath stage.
- d.*—Monaster stage.
- e.*—Monaster stage with nuclear spindle, with polar zones at its extremities (equatorial plate stage).
- f.*—Splitting of chromosomes.
- g.*—Chromosomes, after splitting, proceeding to poles of spindle (metakinesis).
- h.*—Dyaster stage.
- i.*—Convolution stage (daughter cells).
- j.*—Resting stage (daughter cells).

FIG. 2.V.S. EPIDERMIS OF YOUNG SALAMANDER, STAINED WITH
SAFFRANIN \times 300.

- a.*—Superficial cells of epidermis containing pigment.
- b.*—Cell more deeply placed, showing resting stage of nucleus.
- c.*—Convolution stage.
- d.*—Wreath stage.
- e.*—Monaster stage.
- f.*—Dyaster stage.
- g.*—Convolution stage in daughter cells.
- h.*—Pigment cells beneath epidermis.

Fig. 1.



Fig. 2.



respective nuclei, and with them constitute the two daughter cells. But the method of indirect fission is much the more common. In this, as in the case of direct fission, the division of the cell is preceded by that of its nucleus into two daughter nuclei. The protoplasm of the cell becomes constricted, and ultimately divided into two parts at a plane lying between, and we have two daughter cells produced. From the commencement of the division of the nucleus to its completion certain remarkable changes take place in the appearance and arrangement of its chromoplasm, and to these changes the term karyokinesis or mitosis has been applied.

INDIRECT FISSION—KARYOKINESIS.

The changes occurring in a dividing nucleus have been described by Flemming, Rabl and others with great minuteness, but it will be advantageous to refer here only to the more important and obvious alterations, since the average student will have little or no opportunity of observing any but these; and the placing before him of a mass of minute histological detail, the practical study of which only comes within the reach of the specialist, is against the general principle of this work.

Karyokinesis, may be divided into the following stages (*see Fig. 1*).

1.—*Network or resting stage*; 2.—*Convolution*; 3.—*Wreath*; 4.—*Monaster*; 5.—*Dyaster*; 6.—*Wreath*; 7.—*Convolution*; 8.—*Network or resting stage*.

1.—Network or Resting Stage.—As we have seen, this is the inactive condition of the nucleus, as far as division is concerned. It is the condition in which it is found before the process of karyokinesis becomes initiated; and its nature has already been described.

2.—Convolution Stage.—In this stage the nucleus becomes enlarged, its network, nuclear membrane, and nucleoli becoming converted into a skein or convoluted thread. The thread is at first narrow, and the filaments, closely approximated to each other, possess more delicate lateral branches, so that an appearance of more or less density is produced; but this is succeeded by a retraction of the branches, and a thickening, accompanied with a shortening, of the filaments of the skein, by which a more open and simple arrangement results,

enabling the separate portions of the thread to be more clearly seen. At this stage especially the histological characters of the chromoplasm may be studied with a sufficiently high power.

3.—Wreath Stage.—Here the convoluted skein becomes arranged in the form of a wreath or spirem, but its filaments are continuous, and probably still form one continuous thread.

4.—Monaster Stage.—The monaster stage is marked by the breaking of the loops of the wreath at their periphery, so that in place of a thread arranged as a spirem, we have a number of looped **V** shaped filaments, entirely separated from each other, and arranged radially, with the point of the **V** directed to the centre of the nucleus.

About this time or a little earlier, the achromatic “spindle,” composed of delicate achromatic threads arranged in the form from which it derives its name, makes its appearance, and the **V** shaped loops or “chromosomes” as they are called, are arranged around its broadest or equatorial plane in a radial manner. The ends of the achromatic spindle each abut on a central polar particle, from which a further cone of achromatic filaments radiates in the opposite direction into the surrounding protoplasm.

5.—Dyaster Stage.—The next stage, that of the dyaster or double star, is ushered in by a longitudinal splitting of the chromosomes. The splitting commences at the apices of the **V** shaped loops, and extends throughout them, the halves of the split apices becoming turned towards different poles of the achromatic spindle, along the threads of which they travel. As they reach the ends of the spindle they assume a star-like appearance, similar to that of the mother star from which they were derived. Thus, by the process of the splitting of the loops, we have in each daughter star the same number of chromosomes as existed in the mother star.

There is a point of special interest to be noted in connection with the chromosomes. Their number is not accidental, it is special to each species. It may be well, in order to emphasize the importance of this point, to refer here incidentally to what is termed by biologists the process of “reducing division” preceding the fertilised ovum. The sperm mother cell, as it is called, possesses in the case of *ascaris megalocephala* eight chromosomes or **V** shaped loops. When its nucleus divides into two, these do not split longitudinally, so that each daughter cell possesses only four. One of these daughter cells is the first polar body, which is lost. The remaining one again divides, the four chromosomes it possesses again being partitioned in

like manner, two to the second polar body, and two to the female pronucleus.

The male element pursues a similar course, except that there are no polar bodies to be lost. The original sperm cell possessing eight chromosomes divides into two cells, each of which possesses four, and these each divide into two spermatozoa containing two chromosomes each. A spermatozoon containing two chromosomes, after entrance into the unfertilized ovum represents the male pronucleus and fuses with the female pronucleus, to form a nucleus capable of ordinary karyokinetic division, *i.e.*, one in which the V shaped loops or chromosomes split, and so transmit a corresponding number of these to the daughter cell.

6, 7, 8.—The succeeding changes are simple. Each daughter nucleus passes through the first three stages above described, but in the reverse order, until the resting stage is attained, and the process is completed by the division of the protoplasm of the cell itself.

Note.—(a,) In the process of karyokinesis the nuclear wall, as well as the network it encloses, is resolved into the skein or convolution, so that the outline of the changing nucleus is not so evenly defined.

(b,) The change from the network stage to the monaster is accompanied with an appearance of *clearing* of the nucleus, resulting from the threads becoming thicker and shorter, and so allowing the clear nuclear matrix between them to become revealed. The perinuclear protoplasm of the cell also becomes clearer.

(c,) The chromatin of the chromoplasmic fibre is said to be identical with nuclein.

The achromatin has also been called linin. The nature of the nuclear matrix is unknown. The achromatic spindle may be composed of linin.

Examine the following specimen :—

V.S. Epidermis of tail of young salamander, stained with saffranin. B. (Fig. 2.)*

Study the different stages of karyokinesis in the epidermis of young salamanders which have attained a length of from $\frac{1}{2}$ to 1 inch. They may be hardened in Flemming's solution, or in picric acid. When Flemming's mixture is used, the process takes from 6 to 24 hours. At the end of that time wash in water and transfer by gradations to strong spirit. They do not, however, keep satisfactorily for an indefinite length of time.

The sections (*e.g.*, of the tail) should be cut in paraffin, and

* B=Balsam.

stained after they are cut, as the tissue is too delicate for the method of cutting in gum to be available. Fix a section on the slide with shellac and creasote in the usual way, remove the paraffin with turpentine, and the turpentine with alcohol. Now stain with saffranin, which is especially serviceable for nuclei undergoing division.

Saffranin	{ Saturated alcoholic solution	100 c.c.
	{ Water	50 c.c.

An hour or two at least is usually required to stain properly, and sometimes even longer, so that after a few drops of the fluid have been placed on the section, the slide should be removed to a moist chamber in order to retard evaporation. Such a receptacle is readily contrived by inverting a small bell jar over water in a plate or saucer. A metal tray, or anything else which will serve the purpose, is placed in the centre of the plate and bears the slide or slides, which are thus above the level of the water. After staining, subject the section to the action of acid alcohol, in order to remove the superfluous colouring matter from the perinuclear part of the cells, saffranin being a diffuse reagent. A few minutes is usually sufficient to effect this, care being taken that the process is not carried too far. To avoid this risk, absolute alcohol may be used, which acts in the same manner but more slowly. Now dehydrate with absolute alcohol, clarify with cedar or clove oil, and mount in balsam. Clove oil dissolves the saffranin still further; therefore, if sufficiently decolourised previously, it is better to use cedar oil. On the other hand, should the decolourisation have been insufficient, clove oil has an advantage.

Under the low power, $\times 50$, examine the general appearance of the section; if a transverse one of the tail it will be somewhat lozenge shaped, with the acute angles of the lozenge a little drawn out. Note the layer of epidermis surrounding the section. Even under this power the nuclei can readily be seen in various parts, in different stages of karyokinesis. It is immaterial in what order the stages are examined; select a cell showing a monaster, wreath, or dyaster, and put on the high power. Note the general enlargement of the cell as compared with those around it, the clearness of its protoplasm, and of the nuclear matrix; the deeply stained filaments of chromoplasm forming the wreath or star, as the case may be. Follow the epithelium round the section, and in most cases instances of each stage of division will

easily be found. Note how clear the ground substance of the cells has become, which show the mitotic changes. The nuclear spindle is not well revealed by this method of staining.

Instead of a section being made of the tail, or in addition to such a section, a very satisfactory demonstration of mitosis may be obtained by staining with saffranin a thin layer of the epidermis of a salamander hardened in the above manner. The cells are then seen in surface view.

Specimens may be stained in bulk with Kleinenberg's hæmatoxylin. Saffranin, however, usually gives the most satisfactory results.

The Simple Tissues will be considered in the following order:—

The Blood and Lymph.

The Epithelia.

The Connective Tissues.

Muscular and Nervous Tissues.

THE BLOOD.

We have here to deal with one of the few instances of "Cells suspended in a fluid." The cells are termed blood corpuscles and the fluid in which they float the *liquor sanguinis* or blood plasma. The blood corpuscles are of two kinds, the red and the white, of which the former are much the most numerous.

Red Blood Corpuseles.—The red corpuscles are the oxygen carriers of the blood. They acquire oxygen in the lungs, which combines loosely with the hæmoglobin contained in them, and carry it to every part of the body. They differ in size, shape and structure in different animals.

1.—*The red blood corpuscles of amphibians. (Frog.)*—The red corpuscles of the frog are nucleated, elliptical and biconvex in shape, $\frac{1}{1000}$ inch long and $\frac{1}{1700}$ inch broad. Seen singly they are of a pale yellow colour. They possess an elastic envelope and a perinuclear network extending between it and the nucleus—the stroma—the meshes of which contain a solution of hæmoglobin, the colouring matter of the blood. The stroma is said to consist of a combination of certain proteids and fats. The nucleus is oval in shape, and possesses the usual nuclear network. (*Fig. 3.*)

2.—*The red blood corpuscles of birds and fishes.*—These are

nucleated, biconvex and elliptical, but they differ from the amphibian in size and shape. They are both smaller, their length being about the same as the breadth of the red corpuscle of the frog. They differ from each other considerably in breadth, the corpuscles of fishes' blood being broader and in birds narrower, in proportion to their length, than in the case of the frog. (*Fig. 6.*)

3.—*The red blood corpuscles of mammals.*—These differ from those already referred to, in being non-nucleated, bi-concave discs. Though their size varies in different species, their shape is constant throughout the mammalia, except in members of the camel tribe, in which they are elliptical. In man they are about $\frac{1}{3200}$ of an inch (or 8μ) in their broad diameter, and about $\frac{1}{12800}$ in. (or 2μ) in thickness. They possess an elastic envelope and a stroma similar to that of the amphibian corpuscle, containing in its meshes a solution of hæmoglobin. In number they are about 5,000,000 to a cubic millimeter of blood. (*Fig. 4.*)

Blood Crystals.—1.—Hæmoglobin crystals can be obtained by adding a drop of water to some blood on a slide. The hæmoglobin is dissolved out of the corpuscles, and crystallises as the fluid dries. In nearly all mammals the crystals are rhombic. In man they occur as elongated straw coloured prisms. (*Fig. 5 B.*)

2.—Hæmin crystals are obtained by heating blood with a crystal or two of common salt and glacial acetic acid. They occur as rhombic prisms, much smaller than those of hæmoglobin, of a deep reddish brown colour. (*Fig. 5 A.*)

White Blood Corpuscles.—The white corpuscles are much fewer than the red, being in the proportion of about 1 in 500. They are nucleated masses of protoplasm, possessed of the faculty of amœboid movement, and of absorbing into their interior, and digesting, foreign particles with which they come in contact. They may thus be regarded as the scavengers of the body, and it is in this capacity that they absorb bacilli which they meet in the blood stream. In all probability the power of the individual to resist infection by certain diseases, depends on the vital activity of these white cells in destroying the germs, or microbes, as they enter the blood stream. From this function of "eating" foreign particles the cells have been called "phagocytes." In virtue of their amœboid power they are able to change their shape and even their position, so that they may be seen moving across the field of the microscope. The movement may be described as a flowing one; a pseudopodium is thrust out in a certain direction,

it increases in size, and gradually the rest of the cell follows it. They are destitute of ordinary cell walls, and this facilitates absorption of particles into their interior. If the particle is large, the protoplasm may include it by flowing round it. They are viscous, and thus tend to adhere to the vessel wall, or to the cover-glass and slide in a fresh preparation.

There are three varieties described :—

1.—*The Large finely granular Corpuscle.*

2.—" " *coarsely* " "

3.—*The Small white Corpuscle.*

The large white corpuscles are larger than the red, being about 10μ in diameter when round. They often exhibit three nuclei, either entirely separate from each other, or united together by narrower connecting links of nuclear substance (tripartite nucleus), or there may be a single cylindrical nucleus variously coiled. Sometimes the surrounding protoplasm is finely, and sometimes coarsely granular, the difference probably depending on nutritive changes in the cells. The third variety, which may also be finely or coarsely granular, is much smaller than the others, with one comparatively large nucleus and a small amount of perinuclear protoplasm. (*Figs. 3, 4, and 6.*)

Blood Platelets.—In the plasma, in addition to the ordinary red and white blood corpuscles, there are to be seen small discoid or oval, homogeneous, colourless corpuscles, which have been called blood platelets, and also hæmatoblasts. They can be readily made out with a little care. Their nature and significance are not definitely determined.

Fibrin Filaments.—If blood be shed into a vessel, it "clots" in a short time, the fibrinogen in the plasma being precipitated in the form of a network of filaments of fibrin in the meshes of which the blood corpuscles are entangled. In a little time this first clot contracts and expresses the serum of the blood, which is thus composed of the plasma minus the fibrin, while the clot consists of the corpuscles and the fibrinous network.

The same precipitation of fibrin in the form of a delicate filamentous network takes place after a short time in a microscopical preparation of fresh blood; and the net work with its fine filaments, crossing and recrossing each other in every direction, can be readily observed in the fluid serum between the corpuscles.

FIG. 3.

BLOOD OF NEWT, STAINED WITH Picro-CARMINE $\times 300$.

- a.*—Red corpuscles, seen on the flat.
- b.*—Red corpuscle, seen on edge.
- c.*—Large white corpuscles, showing pseudopodia.
- d.*—Blood platelets.
- e.*—Non-nucleated fragment of white corpuscle.
- f.*—Small white corpuscle.

FIG. 4.

HUMAN BLOOD, STAINED WITH Picro-CARMINE $\times 300$.

- a.*—Red corpuscles, seen on the flat.
- b.*—Red corpuscle, seen on the edge.
- c.*—Large white corpuscle—processes retracted.
- d.*—Large white corpuscle—processes extended.
- e.*—Small white corpuscle.

Fig. 3

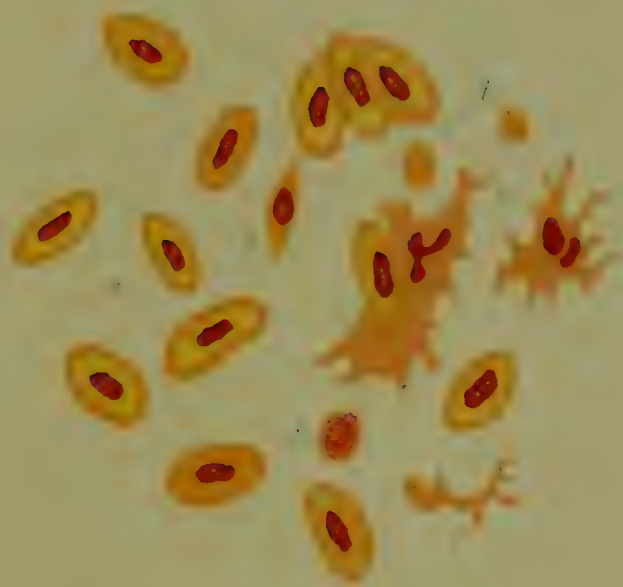
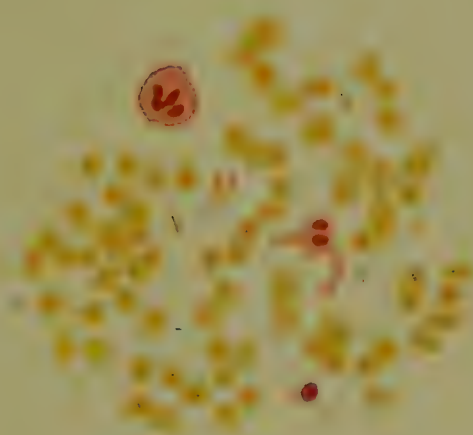


Fig. 4





MICROSCOPICAL EXAMINATION OF BLOOD.

A.—AMPHIBIAN BLOOD.

The structure of amphibian blood corpuscles may be studied either in the frog or the newt. Pith or stun the animal, amputate the long toe of the former, or a short piece of the tail of the latter, and from the stump obtain a small quantity of blood. A sufficient quantity for a preparation can be obtained by pressing the cut surface against the slide. It is not advisable to have a large drop, as the corpuscles are then seen heaped up together *en masse*, and their appearance, and the different varieties of them, are not so easily studied. Put on a cover-glass and examine with the high power. Observe, first of all, the elliptical "red" corpuscles, of a faint yellowish colour. Note, that at first the nuclei are not very apparent, probably from the fact that their refractive index is somewhat similar to that of the rest of the cell. After a short time, however, some change takes place, in virtue of which they become more visible, as colourless, oval, granular bodies, placed in the centre of the corpuscles.

Some of the corpuscles will be seen edgeways; that is, with their narrowest diameter towards the observer. Notice their somewhat spindle shape from this point of view; the pointed extremities of the spindle, with the biconvexity in the centre, due to the presence of the nucleus. Observe the darker colour of the corpuscle, as compared with those seen on the flat. This is due to the fact that when seen in this position there is a greater depth of hæmoglobin for the light to pass through. Observe the transparency of the corpuscles, which is very easily determined in those parts of the specimen where they are more thickly gathered together, and occur in more than one layer; the contour of those beneath being clearly visible through the corpuscles lying above them.

Examine the white corpuscles. Select a large one and note its irregular shape, its granular white appearance, and the presence of one or more nuclei. Make a series of sketches of its outline at intervals of a minute or two. The change in shape is so gradual, that a record of this kind is of great service in enabling one to realize the extent to which it takes place. The blood of the frog or newt is particularly serviceable for the study of amœboid movement, as having been taken from a cold-blooded animal, the hot stage is not required to maintain

the life and activity of the white corpuscle. Then look for other large, finely or coarsely granular, cells, and for the smaller ones with only one nucleus. In all, observe the clear, brightly refractile nucleus or nuclei, with the distinct intra-nuclear network. Observe the small homogeneous blood platelets often collected into groups.

Make the following preparations of Frog's blood.

1.—Magenta Preparation.—Stain the fresh blood of a frog with magenta. A special form of magenta solution should be used for staining blood corpuscles.

The following is the formula :—

Magenta (acetate of rosaniline)	-	-	1 gram.
Rectified spirit	-	-	5 c.c.
Water	-	-	15 c.c.
Glycerine	-	-	20 c.c.

Mix a small drop of blood with a little of this fluid on a slide; cover, and examine with the high power. Note the staining of the corpuscles. The nuclei of both red and white are stained deeply, the rest of the corpuscle only lightly so. This is not a permanent preparation.

2.—Glycerine Preparation.—Preserve the blood of a frog by the method described at page 3, either with a 1 per cent. solution of osmic acid, or with Hayem's fluid. Stain with picro-carmin; mount in glycerine jelly; examine with the high power and note the staining of the nuclei with the carmine, both in the case of the coloured and white corpuscles. The protoplasm of the white corpuscles is stained with the carmine more lightly; the perinuclear part of the coloured corpuscles slightly, both by the carmine and picric acid. Specimens thus prepared are permanent. (*Fig. 3.*)

3.—Cover-glass Preparations.—Place a small drop of frog's or newt's blood on a cover-glass; press another cover-glass over it; slide the two apart between the forefinger and thumb, and allow the film of blood on each to dry. When dry, cover each film with a solution of methyl-blue, and allow it to remain for a few minutes or longer, according to the strength of the solution used; or the cover-glass may be allowed to float, film side downwards, on the surface of the methyl-blue solution placed in a watch glass. After a few minutes take the cover-glass up with forceps, and lave it gently in a bowl of water to remove all the stain, except that which has been absorbed by the film; take up

with blotting paper as much of the water from the glass as possible, without touching the film side, and allow to dry. When dry, mount in Canada balsam. It is convenient to place a small drop of balsam on the dried film side of the cover-glass and place it thus, face downwards, on the slide, applying gentle pressure with a needle, if necessary, to spread it out between the two surfaces.

Examine with the high power. The nuclei of all the corpuscles are stained a brilliant aniline blue, the protoplasm of the white corpuscles is stained more faintly, and the perinuclear portion of the red corpuscles not at all. At first sight only the nuclei of the latter are visible, and it requires a little care to make out the delicate outline of the unstained part of the cell.

A better way is to employ the method of *double staining* when making a cover-glass preparation of blood. After using the methyl-blue and removing the superfluous stain by washing, place the cover-glass in some other reagent, such as eosin, for a few minutes; wash, dry and mount in balsam. Examine with the high power, and observe the nuclei of the corpuscles, both red and white, stained blue, while the perinuclear portion is made pink with the eosin. Many such combinations may be tried, such as methyl-green and eosin, hæmatoxylin and eosin, fuchsin and methyl-blue, etc. Cover-glass preparations vary a little; it is sometimes necessary to make two or three before arriving at a successful result; and the specimens usually vary in quality in different parts. The drying of the cover-glasses may be accelerated by gently moving them to and fro, film side upwards, at some distance above the flame of a Bunsen burner. Care requires to be taken, however, that the cover-glass does not become overheated—the neglect of this precaution is a fruitful source of failure. When successful, cover-glass preparations are amongst the most beautiful which can be made.

Effects of Reagents upon Frog's Blood.

Acetic Acid.—Place a small drop of frog's blood on a slide, cover, and examine with the high power. Now apply acetic acid to the preparation by the method of irrigation. With a glass rod apply at one side of the cover-glass a drop of a 1 per cent. solution of acetic acid, and at the opposite side a small piece of blotting paper. As the blotting paper absorbs the blood at one side, the solution of acetic acid passes under the

cover-glass at the other, and mixes with what is left of the blood. Observe first the effect of the acetic acid on the red corpuscles. They swell up and become spherical, their outline becoming first indistinct and then invisible, due to the decolourisation of the corpuscles; the hæmoglobin diffusing out into the surrounding fluid. The nucleus, on the other hand, becomes much more distinct, and sometimes round instead of elongated. If the solution of acetic acid be sufficiently strong, it invariably assumes a yellow colour, due to absorption of hæmoglobin from the perinuclear portion of the corpuscle. Thus, the latter is completely decolourised from loss of hæmoglobin on the one hand to the plasma surrounding it, and on the other, to the nucleus within it. Examine the white corpuscles. They are globular, swollen, and more transparent, and their nuclei are sometimes slightly stained with hæmoglobin, though to a less extent than those of the red. Look for one of the white corpuscles which is so swollen up that the protoplasm has become separated from what then appears as a very delicate cell wall, leaving a clear space between them. From this it may be deduced that the white corpuscle is surrounded with some very delicate form of envelope, even if it be the merest condensation of its protoplasmic network. Such an assumption does not interfere with the fact that it is capable of amoeboid movement, and the reception of foreign particles, as an envelope of a sufficiently delicate and elastic nature would readily permit of any such change of shape.

Water.—Mix a small drop of frog's blood on a slide with its own quantity of water; cover, and examine with the high power. The red blood corpuscles are swollen up, the hæmoglobin diffuses slowly into the surrounding fluid, and the nucleus becomes spherical. The decolourisation is not so rapid as in the case of acetic acid. Observe the white blood corpuscles: they are also swollen up and globular, but their nuclei, as well as those of the red, are not so distinctly revealed as in the last preparation.

Syrup.—Mix on a slide a small drop of blood with a strong solution of cane sugar; cover, and examine. Many of the corpuscles assume a somewhat leaf-like form, with a fold upon them, at the same time becoming much deeper in colour.

Salt Solution.—On the addition of a 2 per cent. solution of common salt, the red corpuscles show a peculiar irregularity of

outline, as if the envelope of the cell were interrupted or pressed inwards at regular intervals. In human blood this reagent causes "crenation" of the corpuscles.

Tannic Acid.—Irrigate a fresh preparation of frog's blood with tannic acid (5 per cent. solution), and observe that the red corpuscles become globular, and the hæmoglobin is extruded from them, frequently in the form of buds; sometimes it appears as a kind of bleb on their surface, sometimes it seems to surround them in a granular form. The bleb has often a curious semilunar shape, with the concavity of the semilune directed towards the envelope of the cell. It may, however, instead of being extended as a bleb, bud, or granular deposit, be found collected around the nucleus.

In the case of water, acetic and tannic acid, the corpuscles swell up and become globular, due to the imbibition of fluid from without—endosmosis—resulting from the higher density of their fluid contents, as compared with the mixture of liquor sanguinis and water in which they float; on the other hand, when a strong solution of syrup is added to blood, the red corpuscles tend to shrink and assume a more or less shrivelled appearance, through the loss of fluid from exosmosis, their fluid contents having less density than the solution of sugar around them.

B.—MAMMALIAN (HUMAN) BLOOD.

Constrict one of the fingers, *e.g.*, the forefinger of the left hand, by winding round its base a strip of linen or the end of a handkerchief, so as to produce venous congestion of the terminal phalanx. If the finger is too tightly constricted, the flow of both arterial and venous blood is interrupted, and the object of the constriction is lost. With a sharp, clean needle pierce the skin at the root of the nail till a small drop of blood appears. Apply a clean cover-glass to it, and place the latter with the drop side downwards on a slide; or the slide itself may be applied, and the drop of blood on it then covered. Examine with the high power. Observe the numerous red corpuscles, and particularly the way they are placed with reference to each other. In some parts of the field the corpuscles will be seen to be for the most part isolated from each other, and on the flat; but in others they are arranged in the form of rouleaux. They have a tendency to cohere

by their broad surfaces, when they bear a strong resemblance to a pile of coins. Note that the rouleaux may be straight or irregular in outline, that they frequently abut against each other at various angles, and form an irregular network in the field of the microscope. Probably owing to their position between the glass surfaces, the corpuscles are still more or less seen on the flat, the "coins" only overlapping each other to a certain extent. Exactly the same thing happens, if a rouleaux of coins be placed on its side on a table, a book or a piece of board placed over them, and a slight movement from side to side, accompanied with pressure, be made. The coins fall over to one side, partially uncovering each other.

Choose a part of the specimen where the corpuscles have not run into rouleaux, in order to study the appearance of an isolated one. Note that it is discoidal and not elliptical in shape, and that there is no nucleus, although at first sight there is somewhat the appearance of one. At a certain focus, *i.e.*, when on focussing down the corpuscles first come in view—they are seen to have a dark shadowed centre, surrounded by a light peripheral ring. On approaching the lens still nearer to the glass, to focus a lower plane, the centre of the corpuscle appears light and the periphery dark. These changes in appearance are due to the fact that, in the first case, the surface of the thicker margin of the biconcave disc is in focus, and the



FIG. C.—Warm stage for study of amœboid movements—simple form.

concave central thinner portion in shadow; while in the second, when the centre is light, this portion of the corpuscle is focussed, and the surface of the peripheral portion not. Contrast the size of the corpuscles with those of the frog or newt. Find a corpuscle seen edgewise, and note its dumb-bell shape, due to

the biconcavity on each side of the disc. Study the white corpuscles. Observe the different sizes, and notice that at this temperature—the temperature of the room—they are usually round and, unlike those of the frog, do not exhibit amœboid movements. To see these in mammalian blood it is necessary to use the hot stage. This may consist of a strip of tin about eight inches long and three broad. (*Fig. C.*) Mark off three inches from one end, and bend the two parts to an obtuse angle. In the centre of the smaller portion a hole is pierced about the size of an ordinary cover-glass— $\frac{3}{4}$ in. The instrument is used as follows: Let the microscope stand vertically, placing the tin upon its stage, so that the aperture in the three-inch end corresponds with the diaphragm of the microscope, while the five-inch end projects upwards and to the right. Now place a freshly prepared specimen of blood under the microscope as usual, and fasten it to the stage with the tin beneath by means of the clips; place a lighted Bunsen burner under the free end of the tin, so that the flame plays against it; place a small piece of wax, melting at about forty degrees centigrade, on the slide near to the preparation being examined, and continue the heating until the wax melts; the burner can then be withdrawn, and reapplied again when the wax tends to become solid; study the amœboid movement, which very rapidly commences, making sketches of the corpuscle studied from time to time, to compare the changes of shape.

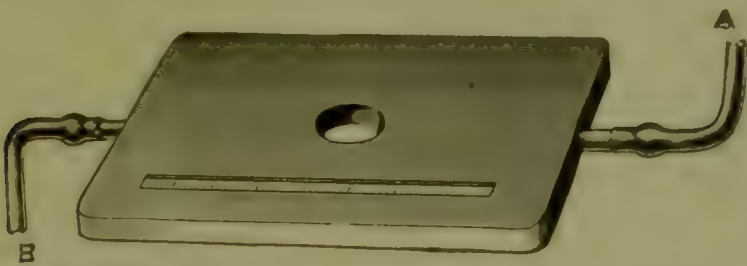


FIG. D.—Warm stage for study of amœboid movements— box form. A—in-flow tube. B—out-flow.

There are other more elaborate forms of the hot stage, as shown above. In these hot water is passed through a brass box, and the temperature is registered by a thermometer in connection with it. The simpler form above described, however, will be found to answer the student's purpose very well.

Make the following preparations of Human Blood.

1.—**Dry Preparation of Blood, Unstained.**—Place a small drop

FIG. 5.

BLOOD CRYSTALS \times 250.

A.—Crystals of hæmin.

B.—Crystals of hæmoglobin.

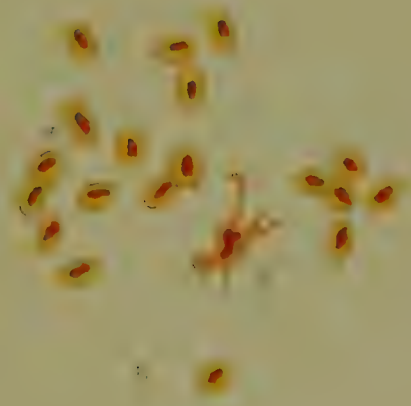
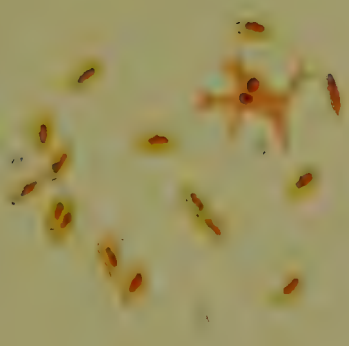
FIG. 6

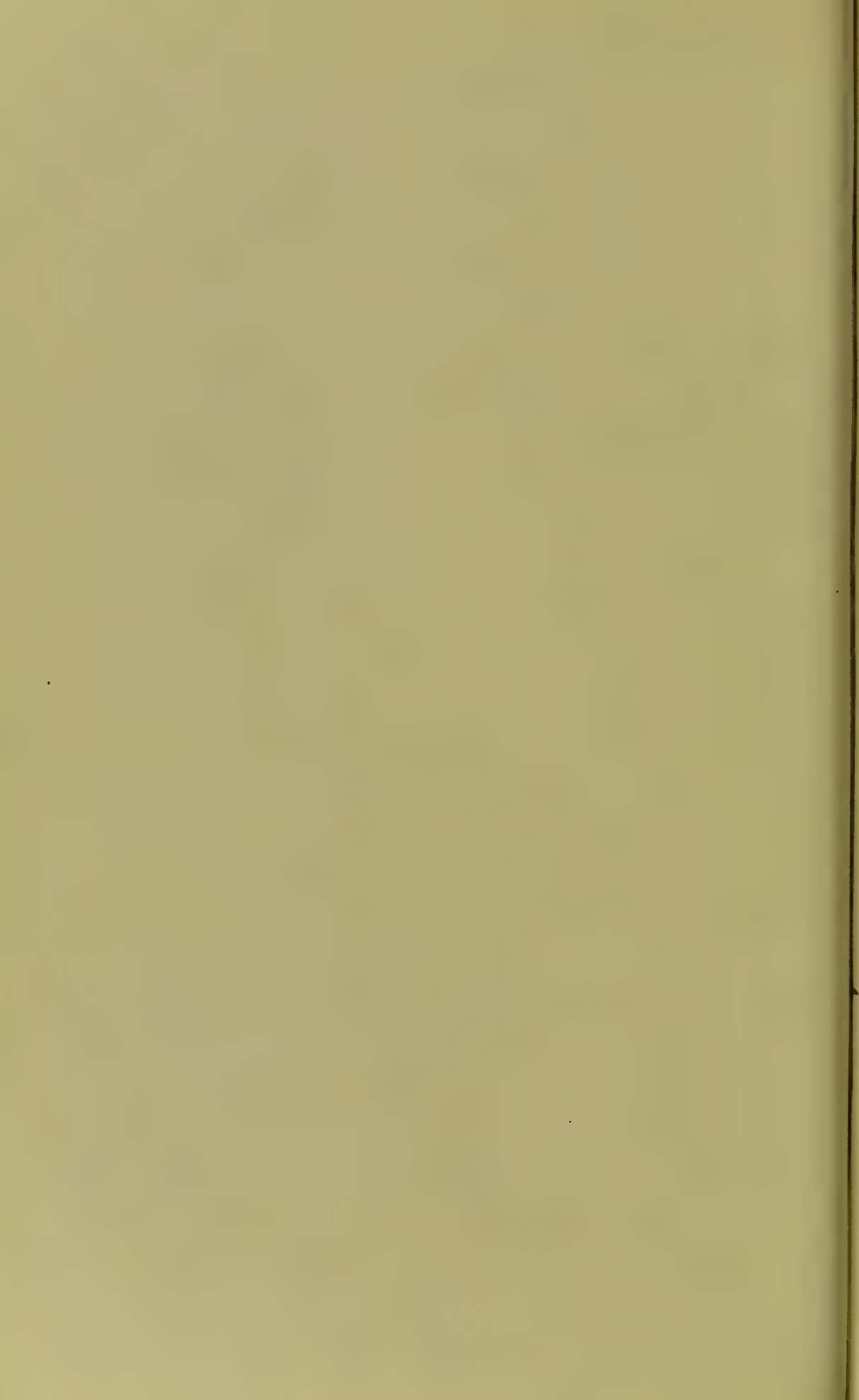
A.—BIRD'S BLOOD, STAINED WITH Picro-CARMINE \times 300.B.—FISH'S BLOOD, STAINED WITH Picro-CARMINE \times 300.*a.*—Red corpuscles.*b.*—White corpuscles.

Fig. 1



Fig. 2





of blood upon a cover-glass, slide another cover-glass over it ; separate them and allow the film on each to dry. The preparation can be mounted by placing the cover-glass film side downwards on a slide, upon which a thin ring of Canada balsam has been previously laid down. Another method is to fix the cover to the slide with a circle of gummed paper about $\frac{1}{8}$ th of an inch broad. Examine with the high power, and note the same characters as described in the last preparation. The white corpuscles are of course fixed in their spherical condition.

2.—Dry Cover-glass Preparation of Blood Stained with Methyl-blue.—Make a cover-glass preparation similar to that of amphibian blood, and study under the high power the red and white corpuscles. In the Canada balsam the red are scarcely perceptible, especially as they have no blue stained nucleus, as was the case with the red blood corpuscles of the frog treated in this way. The white corpuscles are very obvious, however, and constitute the value of the preparation. Note that most of them are round, which is their shape in blood which has been freshly drawn. They are considerably larger than the red corpuscles for the most part, and their nuclei are very beautifully stained. Examine different white corpuscles, in order to see the different forms of nuclei, coiled, distinct from each other, or united with a more or less slender filament.

Effects of Reagents upon Human Blood.

Salt Solution 2%.—Mix on a slide with a small amount of blood, a drop of a 2 per cent. solution of sodium chloride; cover, and examine with the high power. Observe in many parts of the field that what is termed "crenation" of the red corpuscles has taken place. Their surface presents a number of short projecting spines, and a corpuscle consequently bears a certain resemblance to a horse chestnut. This appearance is caused by shrinkage from exosmosis.

Other Reagents.—Apply the other reagents used in examining frog's blood, to human blood, and observe that their effects are essentially the same, but modified by the structural differences between the two.

Blood Crystals, Fibrin Filaments and Platelets.

Hæmoglobin Crystals.—Defibrinate some rat's blood by whipping a small quantity of it in a watch glass with a thin glass rod.

Mix a drop with water on a slide and set aside for a few minutes, and then examine with the high power for crystals of hæmoglobin in the form of rhombic prisms of a faint yellow colour; crystallisation commences first at the edge of the cover-glass. Compare the size and colour of the crystals with those of the following.

Hæmin Crystals.—Add a crystal or two of common salt to a little powdered dry blood on a slide; add a small drop of glacial acetic acid, and cover. Warm the slide over a Bunsen flame, till small bubbles of gas can be seen rising beneath the cover-glass. Examine with the high power. As the preparation cools, small dark reddish prisms of hæmin crystallise out.

Fibrin Filaments.—Mix a small drop of blood with a little 6 per cent solution of common salt on a slide; cover, and set aside for an hour or two to allow the blood to coagulate. Then wash the preparation with a weak solution of alcohol, by the method of irrigation, whereby the red blood corpuscles become decolourised. Introduce a solution of Spiller's purple by the same process; the fibrin filaments will be stained blue. With the high power observe the network of fibrin filaments, which frequently seem to radiate from the blood platelets.

Blood Platelets.—Mix a small drop of blood, obtained in the usual way from the finger, on a slide with an equal quantity of Bizzozero's fluid (methyl-violet 1 part in 1000 of water), and cover. Under the high power note that the red corpuscles are unstained, but that the white and the blood platelets are coloured by the reagent. Look for the latter as small homogeneous discoid particles often collected into groups.

THE LYMPH AND CHYLE.

Lymph is composed of a plasma almost identical in nature with that of blood, and contains a number of cells which are called lymph corpuscles. These are, many of them, similar to the white blood corpuscles. Very many are small cells possessing one nucleus, and very little perinuclear protoplasm, which have evidently been derived from the lymph glands, the lymph coming from these containing a greater number of such corpuscles than that going to them.

Chyle, the fluid found in the lymphatics, coming from the intestine, is of the same nature as ordinary lymph, but contains

in addition absorbed products of digestion. Amongst these are numerous globules of fat of various sizes, to the presence of which its milky appearance is due.

DEVELOPMENT OF BLOOD CORPUSCLES.

Origin of the White Blood Corpuseles.—The white corpuscles of the blood are undoubtedly produced in the lymphatic glands and collections of lymphoid tissue generally throughout the body, after these organs have been developed. In the lymph nodules a process of karyokinesis is continually going on during life, and the corpuscles thus produced join the lymph stream and are ultimately poured into the blood at the root of the neck. In early embryonic life however, the first white corpuscles appear to be modified mesoblastic cells, which enter the blood stream through the walls of the vessels by virtue of their capacity for amœboid movement.

The white corpuscles produced in the mesoblast before the appearance of the lymph nodules enter the blood stream in a similar manner to those produced later, but by a less circuitous path. They traverse the lymph spaces in the tissue between them and the vessel wall, which they pass through in virtue of their amœboid faculty of movement. Those produced later in the lymph glands and nodules follow a more elaborate system of channels to the vessels at the root of the neck. (*See chapter on "Lymphatics."*)

At the same time it must be remembered that, throughout life, the leucocytes or wandering cells in ordinary connective tissue can pass through the capillary walls from the tissue to the blood, and the leucocytes or white corpuscles of the blood directly into the tissues outside it.

Origin of the Red Blood Corpuseles.—The manner in which the red blood corpuscles are developed cannot be said to have been yet determined with any real accuracy. That they are being continuously destroyed and renewed during life, just as the cells of the tissues generally, may be accepted with certainty, but the exact *modus operandi* of either process is still in doubt. The earliest red corpuscles in the mammalian embryo appear to be formed at the same time as the first blood vessels, in the following way: Certain mesoblastic cells ("angio-blasts," or nucleated masses of undifferentiated protoplasm)

branch and anastomose with each other to form a protoplasmic network.

The nuclei of these cells multiply and gather together, especially at the nodes of the protoplasmic network, where they form collections or "nuclear nests." Some of the nuclei remain, however, in the strands of the network. The protoplasm around the nuclei in the central portion of the nests undergoes a change by which it resembles a stroma containing hæmoglobin in its meshes. The small amount of protoplasm remaining between the individual nuclei with their investment of hæmoglobin containing stroma, undergoes solution, and we now have coloured, nucleated corpuscles floating freely in a cavity; which extends by the same process of protoplasmic solution along the strands of the protoplasmic network, till a system of intercommunicating vascular canals is formed. The walls of these canals are formed of flattened nucleated cells, derived from the remaining undifferentiated protoplasm of such of the nuclei as have not taken part in the formation of coloured blood corpuscles. Later, in mammalian embryonic life, these nucleated coloured corpuscles disappear and are replaced by non-nucleated coloured discs. These appear to be formed in much the same way as the nucleated ones, except that there are no collections of nuclei to form nuclear nests, nor do any nuclei take part, apparently, in the formation of the corpuscles. (*Schäfer: Subcutaneous tissue of new-born rat.*) Small spheroids containing hæmoglobin appear in the angioblastic cells, and, by the subsequent solution of such of the protoplasm as remains between them, float free in a cavity as before. The nuclei of the cells appear to become merely the nuclei of the epithelial plates of the vessel wall. The strands of the protoplasmic network become hollowed out by solution of the central part of the protoplasm forming them, and bring the cavities containing the young blood corpuscles into communication with each other in the manner already indicated.

This latter method of development of the red blood corpuscles ceases in most animals before birth, and in all shortly after, and both during growth and adult life some process other than intracellular probably takes place. It seems to be generally conceded that this process, whatever its nature, takes place in the red marrow of bones, but the exact details are not yet made out. In the red marrow we have a tissue composed of a network of wide

intercommunicating capillary channels, and between them a considerable number of what are termed "marrow cells." As to the nature of these, we need only say here that in the main they are of the nature of the white corpuscular elements of the blood, and that they vary very considerably in size. Among them however, and in the vascular channels too, are to be found different cells, termed erythroblasts, which are smaller than the marrow cells proper, and resemble the nucleated coloured corpuscle of the embryo. These cells are amœboid as far as movement is concerned. We cannot yet say by what process they become transformed to non-nucleated discs before they enter the general blood stream, nor can we speak definitely as to their immediate antecedents. They may either be derived from the colourless leucocytic marrow cells by the acquisition of hæmoglobin, or they may be developed by simple karyokinetic division of similar pre-existing nucleated coloured cells, direct descendants of those found in the embryo.

There is no satisfactory evidence as yet, that red blood corpuscles are formed from white, nor that they are formed in the spleen.

According to Hayem and Norris, the blood platelets are to be regarded as developing red corpuscles, but the matter requires further investigation.

APPENDIX TO CHAPTER III.

STUDY OF CIRCULATION IN THE TONGUE OR FOOT OF FROG.

Use a strip of thick cardboard 6 inches by $2\frac{1}{2}$ inches. For the tongue it should be perforated with a round hole towards one end ; for the foot one end may be notched in a V-shaped manner.

The Tongue.—Pith the brain of the frog the night before. Wrap the body in a piece of moist rag, leaving the head free, and place it on the cardboard so that the tongue, when drawn from the mouth and pinned out, will extend over the aperture in the board. The animal should be placed on its back, so that the under surface of the tongue will be uppermost. Pin out the tongue, add a drop of water, and cover. Place the board on the stage of the microscope so that the tongue is over the aperture of the diaphragm.

The Foot.—The same method is adopted in the case of the foot, but the cardboard notched at one end is used instead. Pith the brain, wrap all but one leg of the animal in a moist cloth, and extend it upon the cardboard so that the web of the foot is over the V-shaped notch. Attach threads to two of the toes, and adjust the web between them by carrying the threads through slits in the cardboard on either side of the space. Add a small drop of water, cover, and place under the microscope as before. A fragment of a cover-glass is usually large enough to serve the purpose ; if a whole one is used it is raised by the bony projections of the phalanges above the web.

Under the high power study the circulation in the arteries, capillaries and veins. Observe the very rapid flow in the arteries as contrasted with that in the veins, their narrower diameter, and the fact that the blood moves from the large to the smaller vessels, but *vice versa* in the veins. In the latter, where the current is slower, endeavour to distinguish a central or axial stream of red corpuscles, and a peripheral one, moving more slowly, in which the white corpuscles are especially to be seen in close contact with the vessel wall. Observe the way in which the white corpuscles progress. Their viscosity makes them tend to stick to the wall of the vessel, and advance by a rolling movement upon it.

Find a capillary and study the red corpuscles. Note how readily their elasticity enables them to change their shape, either in response to mutual pressure, or to enable them to pass through a narrow channel. Find a spot where the capillary bifurcates, and observe the behaviour of a red corpuscle when it is caught on the bifurcation. It bends over to each side, and remains for a short time accurately balanced, until another corpuscle strikes it on one side, or in some other way the balance becomes destroyed, when it rapidly shoots down the stream in one of the two branches, regaining its original shape immediately it is released from its position in the fork.

After pithing, the frog may be curarised in order to ensure perfect quiescence, but it is apt to affect the calibre of the vessels. As a rule, if the animal is suitably wrapped up, reflex movements do not take place when it has once been fixed in position on the stage of the microscope.

CHAPTER IV.

*THE SIMPLE TISSUES (continued).**EPITHELIUM—ORDINARY CONNECTIVE TISSUE.***EPITHELIUM.**

THE varieties of Epithelium belong to that class of tissue which has been defined as consisting of "cells arranged on a free surface." The cells are merely cemented together, and there is no other intercellular matrix.

Epithelium is found constituting the epidermis of the skin ; lining mucous membranes, such as the alimentary, respiratory, and genito-urinary ; serous cavities, such as the pericardial, pleural and peritoneal sacs ; the heart, blood and lymphatic vessels ; forming the alveoli or tubules of secreting glands and the ducts passing from them ; lining the central canal of the spinal cord and the ventricles of the brain ; constituting special nerve terminations, etc.

Epithelium may be classified according to its ancestry, function, or morphological characters.

I.—ANCESTRY. The epidermis and lining membrane of the central neural canal are of epiblastic origin.

The epithelium of the alimentary canal, of the glands opening into it, and of the air vesicles of the lung, is derived from the hypoblast.

The peritoneal epithelium, and that of other serous cavities, of the vascular and lymphatic systems, is from the mesoblast.

II.—FUNCTION. Epithelium may be mainly *protective*, as in the case of the skin. It *diminishes friction* between surfaces which are intended to glide smoothly over each other, as when it forms part of the serous investment of the heart, lungs, and other viscera, the synovial membranes of joints, the visceral

FIG. 7.

SUPERFICIAL LAYER OF EPIDERMIS OF NEWT, STAINED WITH
HÆMATOXYLIN $\times 250$.

- a.*—Squamous cell.
b.—Nucleus.

FIG. 8.

A.—SQUAMOUS EPITHELIUM LINING ARTERY, STAINED WITH NITRATE
OF SILVER $\times 250$.

B.—SQUAMOUS EPITHELIUM OF OMENTUM OF RABBIT, STAINED WITH
NITRATE OF SILVER $\times 250$.

- a.*—Silvered outlines of cells.
b.—Nucleus.

Fig. 7.

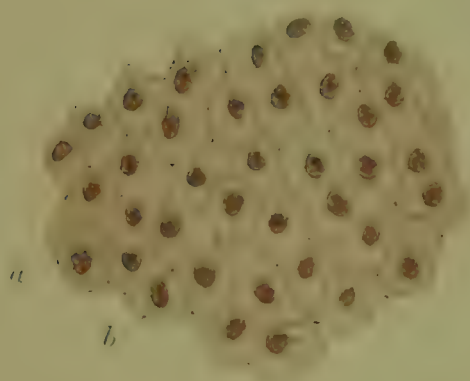
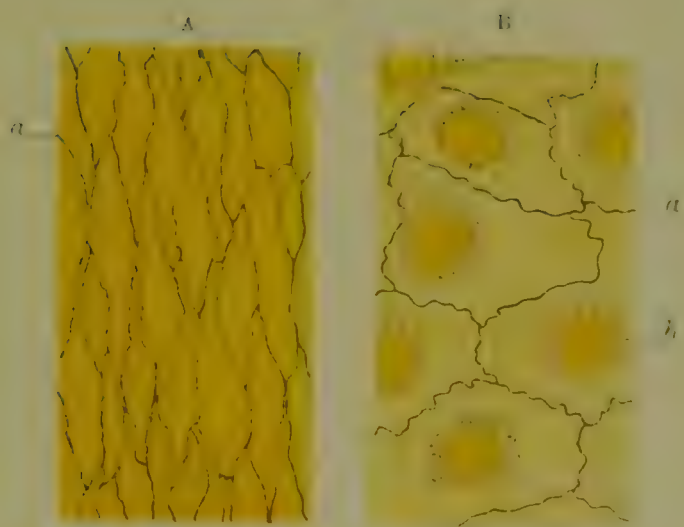


Fig. 8.



and parietal layers of the conjunctiva ; and also between the blood and the vessels through which it passes by affording an exceedingly smooth lining to their walls. Where it lines the alveoli of the lungs it forms a thin membrane, which readily *permits of an exchange of gases*, between the blood on one side of it, and the air on the other. It may be *secretory*, as in the case of the salivary glands, liver, and pancreas ; or it may have the faculty of *absorption*, of which the epithelium of the intestinal villi is an example. It has the power of *moving fluid over its surface* when, as ciliated epithelium, it lines the trachea, Fallopian tubes, and epididymis. Its function may be purely *nervous*, as when it forms the terminations of sensory nerves, such as those of hearing, taste, and smell.

III.—MORPHOLOGICAL CHARACTERS. These, with which the histologist is mainly concerned, afford the following classification :—

- 1.—*Squamous. (a. Simple. b. Stratified.)*
- 2.—*Columnar.*
- 3.—*Ciliated. (a. Simple. b. Stratified.)*
- 4.—*Transitional.*
- 5.—*Glandular.*

1.—**Squamous Epithelium.**—This epithelium is simple or stratified, that is, it occurs either as a single layer of flattened cells or as several superimposed layers, in which case only those near the surface are composed of squames.

a. Simple Squamous Epithelium has for its function in many places the reduction of friction to a minimum between surfaces working over each other. Thus, it is found lining the peritoneal cavity, affording a covering to the organs which the peritoneum invests, and permitting them to move freely upon each other. For the same purpose it lines the pericardial and pleural sacs, and, as already stated, it affords a smooth lining to the heart and blood-vessel walls. Where it lines the alveoli of the lungs, and forms the walls of the blood capillaries, it has another function, that of permitting an interchange of gases between the blood and the air in the one case, and the blood and the tissues in the other. It is also found covering the posterior surface of the cornea, the anterior surface of the iris, and lining other lymph spaces and channels.

Simple squamous epithelium consists of a single layer of flattened nucleated cells, which vary in shape according to the

situation in which it occurs. The outline may be polygonal, fusiform, sinuous, etc. The cells are accurately fitted to each other by their edges with cement substance, which can be stained black with nitrate of silver.

Examine the following specimens :—

(1,) *Moult from skin of frog or newt stained with hæmatoxylin. F. or B. (Fig. 7.)*

With the high power observe the polygonal shape of the cells, the outlines of which can in this specimen be easily seen without the assistance of silver nitrate. Each cell possesses a round nucleus stained deeply with the reagent. The protoplasm of the cells is granular, especially around the nucleus.

(2,) *Omentum of cat stained with nitrate of silver and hæmatoxylin. F. (Fig. 9.)*

The great omentum is the double fold of peritoneum depending from the greater curvature of the stomach. It consists of a coarse network of white fibrous tissue, the trabeculæ of which are completely invested with a single layer of squamous epithelial cells. Examine the specimen first under the low power. Recognise the network with the meshes varying in shape and size in different parts, and the blood-vessels, often accompanied with fat cells, passing here and there through the tissue. In their immediate neighbourhood the membrane ceases to be fenestrated, and consists of a continuous layer of connective tissue, with epithelium on either side. Select a fenestrated portion, and examine with the high power. The meshes or areolæ of the network are much broader, as a rule, than the trabeculæ bounding them. Study particularly the epithelial cells lying on the surface of, and investing, the fibrous trabeculæ, noting their varying outlines stained black with the silver nitrate. Each cell contains a round or oval nucleus, stained blue with the hæmatoxylin. Distinguish between these and the connective tissue cells situated between the fibres of the trabeculæ. The former are usually round, and placed near the centre of the cells to which they respectively belong; the latter are entirely within the epithelial covering; they are fusiform in shape, and run in the long axis of the trabeculæ between the bundles of fibres forming them.

(3,) *Small intestine of rabbit injected with nitrate of silver. B. (Fig. 10.)*

With the low power, first note the complicated network of

vessels outlined with the silver nitrate. Select a thin portion of the specimen, one which transmits light readily, and put on the high power. It is not usually difficult to find, in several parts of such a specimen, small arteries and veins running side by side, with the outlines of their epithelial cells revealed by the nitrate of silver. Notice the comparative breadth of the vein, and contrast the epithelium of the two. In both, the cells are more or less elongated, pointed at their ends, and their outline is a little sinuous; but those of the vein are considerably broader and also shorter than those of the artery. The nuclei are unstained. The transverse lines crossing the vessels are due to the reduction of the reagent by the cement substance between the transversely arranged fibres of non-striped muscle which form part of their wall. Examine the epithelium of the capillaries, and note the absence of the transverse lines.

Fig. 65 shows the epithelium forming the wall of a lymphatic vessel, commencing in the central tendon of the diaphragm of a small mammal. Note particularly the extreme sinuosity of the outlines of the cells, stained with nitrate of silver.

b. Stratified Squamous Epithelium is mainly protective in its function. It is found constituting the epidermis of the skin, lining the mouth and the first part of the nostrils, the lower part of the pharynx, the œsophagus, the vagina, the lower half of the cervix uteri, the anterior surface of the cornea, and the two layers, visceral and parietal, of the conjunctiva.

It does not, however, show precisely the same characteristics in all these places, being more complex in its arrangement in the case of the skin, for instance, than elsewhere. As a rule, there are many layers of cells, only the superficial of which, however, are squamous in shape. These are flattened, polygonal in outline, and overlap each other, in this way differing from the squames which occur as a single layer. The nucleus may or may not have undergone change, but the protoplasm of the cells has usually become converted to a homogeneous, horny material termed keratin. The deepest cells of all are much smaller, nucleated, somewhat columnar in shape, and are placed vertically upon a basement membrane, or the condensed surface of the subjacent connective tissue. The cells of this layer are frequently called germinal, as they supply by their repeated division the place of those removed from the surface by attrition. Between them and the superficial squames are several layers of cells of

intermediate character, larger than the germinal cells, nucleated, and polygonal. In many situations, especially in the skin, the margins of adjacent cells of this layer are not in close apposition, but united to each other by intercellular ridges. When the cells are artificially separated from each other, these ridges break in their middle, and the cells appear surrounded with short prickles; they have hence been termed "prickle cells." Between the ridges is a system of intercellular canals, through which lymph readily percolates for the nutrition of the epithelium.

Examine the following specimens:—

(1.) *Isolated superficial cells from epithelial lining of mouth.*

With the finger-nail gently scrape the roof of the mouth so as to detach a few of the surface squames, and examine in normal saline or saliva itself. Under the high power, note the character of the cells. They are large, colourless, roughly polygonal in shape, and flat; they frequently have a nucleus, and the perinuclear part of the cell may be granular. In many cases, however, the cell contents have been converted entirely to keratin. These cells, when *in situ*, are united together by cement, and are not placed edge to edge, but overlap each other. Obtained in this way, they are quite superficial, and a vertical section of the palate is required to show the nature of those situated more deeply.

(2.) *V.S. Hard palate of cat, stained with picro-carmin.*
F. (Fig. 11.)

Place the slide under the microscope so that the surface of the palate is away from you and the deeper part towards you.* Under the low power, distinguish, first of all, the epithelium from the connective tissue upon which it rests. The former is stained both by the carmine and picric acid, and so has a decidedly yellowish tinge; the latter is stained chiefly by the carmine, and is pink in contrast. Observe the two layers of epithelium: a superficial one, especially stained by the picric acid forming a yellowish band along the surface, the stratum corneum, or horny layer; and a deeper, broader one, stained also with the carmine, the stratum Malpighii. Beneath this, note the pink fibrous tissue running up into papillæ between the downward prolongations of the epithelium. Put on the high power, and

* That is, when looked at through the microscope. It must always be borne in mind that the microscopic image is inverted.

study first the stratum corneum. It presents, to some extent, an appearance of longitudinal striation, due to its being composed of superimposed layers of flattened squames, similar to those already examined. Nuclei, rod-shaped or oval in section, are to be seen here and there. Examine the cells of the deeper or Malpighian layer. Observe that their shape and size vary at different levels; the deepest, those immediately above the fibrous tissue, are small and columnar, with elongated oval nuclei; they are the "germinal" cells, which divide to compensate for the loss of those removed by attrition at the surface. Observe too, that above this layer the cells gradually increase in size, become polygonal in outline, with round nuclei, and exhibit the characteristics of prickle cells. The student cannot very well distinguish the intercellular ridges with a power of 300 or 350, but he will, at all events, be able to see that the outlines of these cells are indicated by what appears more like a dotted line than a continuous one. The dots correspond to the lymph channels, and the parts between them to the connections between the cells. On passing to the upper part of the stratum Malpighii, observe that the cells become somewhat flattened, though not to such an extent as those of the stratum corneum. Note the sudden transition from the one stratum to the other. In the skin, too, there is a sharply marked off horny layer, but not in the epithelium covering the cornea.

Examine the connective tissue on which the epithelium rests, and recognise in it the strands of white fibrous tissue, stained pink, with the nuclei of connective tissue cells amongst it, stained deeply with the carmine.

2.—Columnar Epithelium.—Columnar epithelium is found lining the alimentary canal below the œsophagus, and the ducts of the glands opening into it. It is found also in other special situations which will be referred to in the course of the text. Where it lines the small intestine it is mainly absorptive in function, only a few of the cells being modified to secrete mucin. It always occurs as a single layer.

The cells vary in shape in different situations. As seen in a vertical or transverse section of a villus of the small intestine, they are tall and columnar in shape, broader at their free ends than at their attached extremity, and are placed palisade-wise vertically to the surface of the basement membrane on which they rest. Each cell possesses a cell envelope, perinuclear pro-

FIG. 9.

OMENTUM OF CAT, STAINED WITH NITRATE OF SILVER AND
HÆMATOXYLIN $\times 300$.

- a.*—Silvered outline of epithelial cell.
- b.*—Nucleus of epithelial cell.
- c.*—Connective tissue cell of fibrous basis.
- d.*—Connective tissue forming the trabeculæ.

FIG. 10.

BLOOD VESSELS OF MUSCULAR WALL OF SMALL INTESTINE OF
RABBIT, STAINED WITH NITRATE OF SILVER $\times 250$.

- a.*—Small artery.
- b.*—Small vein.
- c.*—Capillary.
- d.*—Lymphatic vessel.
- e.*—"Cement" lines between the muscular fibres of the vessel walls.

Fig 9.

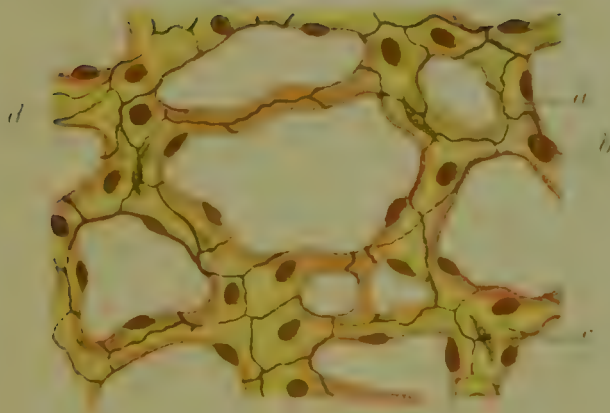
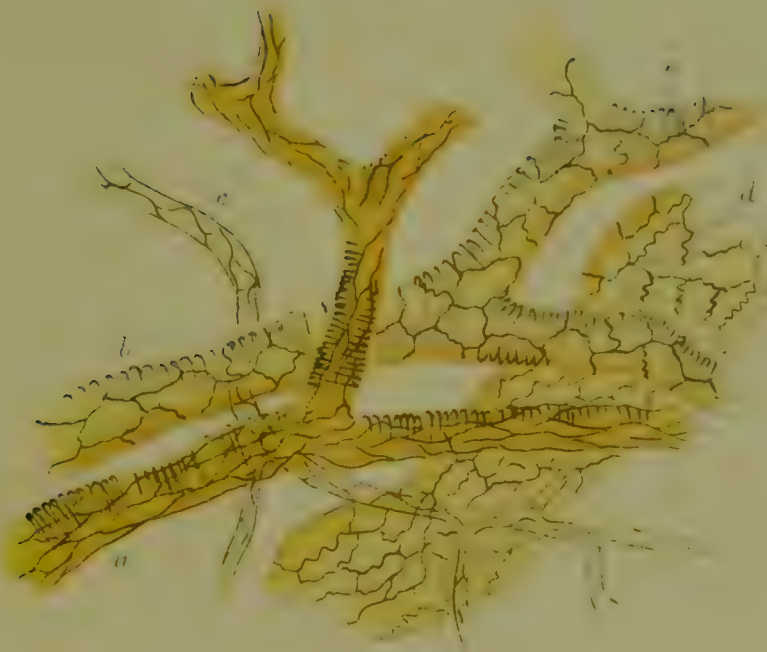


Fig. 10.



toplasin, and an oval nucleus placed towards its attached end. In order that adjacent cells may adapt their contour to that of each other, their nuclei are not usually quite in the same plane. Each cell, at its free border, exhibits what is known as a "striated hem," and this, when the cells are *in situ*, gives rise to the appearance of a bright refractile band lying on the surface of the layer. Here and there among the columnar may be seen goblet cells. The mouth of the goblet cell is at the surface of the epithelium; the stalk is attached to the basement membrane on which the cells are placed. These goblet, or chalice cells, are formed from the columnar by the expansion of the outer portion from the conversion of its contents to granules of mucinogen, and the loss of the striated border. The nucleus, surrounded by the remains of the protoplasm, persists in the stalk of the cell. When isolated from each other, the columnar cells of an intestinal villus are often seen to have lateral, wing-like expansions proceeding from them, and their attached pointed end is not unfrequently irregularly formed.

If the villus is seen from the surface instead of in section, we observe a mosaic of polygonal areas, the lines indicating the outlines of the mutually compressed cells. Here and there a circular opening may be seen, taking the place of one of the polygons. This is the mouth of a goblet cell. On focussing downwards, another circle, rather larger than the first, may come into view, due to the plane of the broadest part of the body of the cell being then in focus.

The columnar cells of the stomach are rather different from those of the intestine. Their free extremities present no striated hem, nor are they goblet-shaped, though their outer two-thirds are clear, not granular, and they secrete mucin. The nucleus, with a little protoplasm, is situated at the attached end of the cell.

The cells lining the ducts of glands vary a good deal in different situations. In some cases they are more cubical than columnar in shape, and possess a round nucleus placed in the middle of the cell. When columnar they are often broader at their attached than at their free ends, due to the fact that when lining a duct they are covering a concave as opposed to a convex surface. The cells of the inter- and intra-lobular ducts of the salivary glands are striated vertically in their outer half.

Examine the following specimens :—

(1.) *V.S. Small intestine of cat, stained with hæmatoxylin. F. or B. (Fig. 12.)*

With the low power, select one of the villi, cut vertically; centre a part of the epithelium covering it, put on the high power, and examine. Determine first, some general points about the villus; the lacteal in its centre and the fibres of non-striated muscle running longitudinally outside it; the delicate adenoid tissue extending from the lacteal to the base of the epithelium, with the lymph corpuscles in its meshes. The capillary network can be seen better in an injected specimen. Now study the epithelium. The outlines of the individual cells will not be well seen if the section is thick, but in a thin one they are quite apparent. Observe the deeply stained oval nuclei placed towards the bases of the cells, and note that those near to each other are frequently on different levels in their respective cells. In addition to these, look for other nuclei placed here and there between the cells. They are those of lymph corpuscles, which have come from the interior of the villus, passed through the basement membrane, and made their way between the cells of the epithelium. In consequence of the presence of these, the columnar cells, when isolated, often show a depression on one side, corresponding to the position occupied by one of them. In addition to this, their lateral contour is often irregular, as already stated, the result of compression by their neighbours. Observe the striated border. Note that the striæ, or rods, run vertically to the surface of the epithelium.

The goblet, or chalice cells, are easily recognised as somewhat egg-shaped spaces apparently interrupting the continuity of the epithelial layer. Distinguish their pointed basal extremity containing the nucleus and the remains of the protoplasm.

Find a villus, or part of one, seen in surface view, and study the polygonal outlines of the columnar cells and the circular mouths of the chalice cells. Focus one of the latter at more than one level, and endeavour to make out two circles, one corresponding to the mouth of the cell and the other to its broadest diameter.

(2.) *Isolated columnar cells from small intestine of newt, stained with picro-carmin. G. J.* (Fig. 13.)*

Examine under the high power, and with the tube of the microscope drawn out. Observe the very various shapes and sizes of the epithelial cells, the large nuclei with their nucleoli,

* Glycerine Jelly.

the striated border, and the irregularity in shape of the narrower attached extremity. In some, it will be seen divided into two processes, in others, giving off lateral wing-like expansions. Look for chalice cells, and note again the great variety in shape, the absence of the striated border, the open mouth, the nucleus at the base of the cell, surrounded with a very small amount of protoplasm, and the clearness of the rest of the cell. In this part the original protoplasm remains as a very delicate network with large meshes containing granules of mucinogen, which become converted to mucin by the action of water.

3. -Ciliated Epithelium.—Ciliated epithelium is found lining the respiratory portion of the nasal mucous membrane, the lachrymal sac and duct, the respiratory portion of the pharynx, the greater portion of the middle ear, the Eustachian tube, the larynx (except the vocal cords), trachea and bronchi (except the smallest or bronchioles), the epididymis and vasa efferentia, the Fallopian tubes and uterus, the central canal of the spinal cord, and the ventricles of the brain (except the fifth).

The function of ciliated epithelium, in mammals, is to move fluid over its surface in a certain direction. Thus, in the trachea and bronchi the mucus secreted by the membrane lining them is caused to flow, along with any foreign particles it contains, upwards towards the pharynx; in the Fallopian tubes the ovum is directed towards the uterus; and in the epididymis the secretion of the testicle is moved towards the vesiculæ seminales.

It may be *simple* or *stratified*, *i.e.*, be composed of either one or more layers of cells. In the central canal of the spinal cord, the ventricles of the brain, the epididymis, the Fallopian tubes and uterus, it exists as a single layer; in the respiratory system, other cells of a germinal and intermediate nature are placed beneath and between the superficial ciliated ones.

A typical cell has the following characters. In shape it is columnar, broad at its free extremity, and tapering towards its attached end. The latter may be branched, as in the trachea, where there is more than one layer of cells. Each cell has an oval nucleus placed, sometimes in its middle, and sometimes towards its base. The free end is covered with a bright refractile disc, and upon this the cilia, or hair-like processes, are placed. They are flattened, apparently structureless filaments, pointed at their free ends, about $\frac{1}{1000}$ of an inch long, and number 10 or 20

FIG. 11.

V.S. STRATIFIED SQUAMOUS EPITHELIUM OF TONGUE OF
RABBIT, STAINED WITH PICRO-CARMINE $\times 250$.

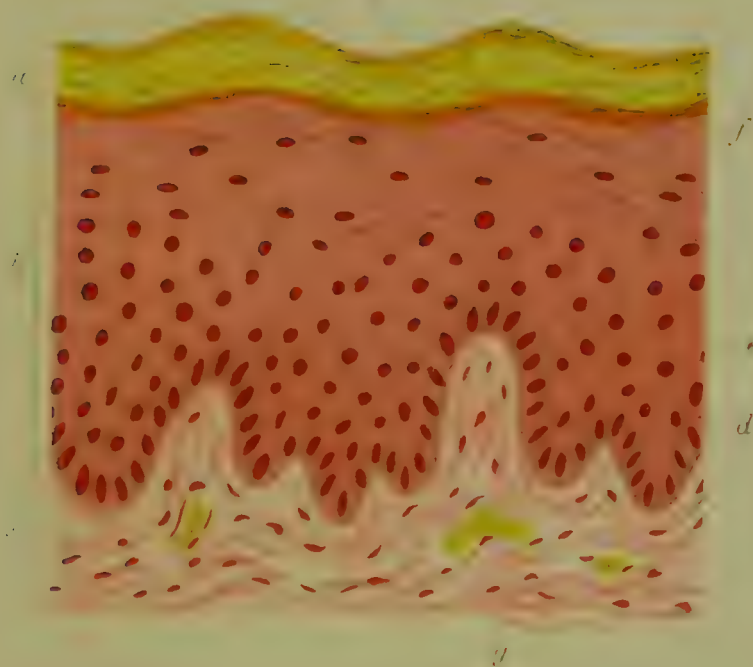
- a.*—Corneal layer.
- b.*—Malpighian layer.
- c.*—Fibrous tissue forming papillæ.
- d.*—Germinal cells.
- e.*—Prickle cells.
- f.*—Cells of stratum Malp. becoming flattened.
- g.*—Blood vessel.

FIG. 12.

V.S. VILLUS OF CAT'S INTESTINE—INJECTED, STAINED WITH
HÆMATOXYLIN $\times 300$.

- a.*—Layer of columnar epithelial cells.
- b.*—Interior of villus.
- c.*—Striated hem of epithelial cells.
- d.*—Adenoid reticulum.
- e.*—Mucous cell.
- f.*—Watney's node.
- g.*—Network of capillaries filled with red injection.
- h.*—Central lacteal of villus.
- i.*—Strand of non-striped muscle fibres.
- k.*—Nuclei of basement membrane.

Fig. 11.



P. 12.



to each cell. Their size and number, however, vary greatly in different animals and also in different situations in the same animal.

In the mussel, each cilium is said to be attached by a narrow neck or "intermediate segment" to a "basal knob." These knobs by their lateral apposition in the same plane, cause the appearance of the bright refractile border. From each knob a varicose filament passes down through the protoplasm of the cell, avoiding its nucleus, to the base, where it unites with the rest to form a narrow leash.

As seen under the microscope, ciliary movement has been likened to the bending of a field of corn in the wind. It consists of the rapid flexion of the filaments in one direction followed by a slower recoil. The first is due, probably, to the possession of contractile power by one side of the filament, the latter to the elasticity of the other. The cilia require for this contraction certain conditions of temperature, moisture, and oxygen.

The action of the cilia is dependent on their connection with the cells to which they belong, but independent of any central or local nervous influence. They may be entirely separated from nerve influence, but provided they are still attached to their cells, the movement continues. It even persists after the nucleus, with the lower part of the cell, has been removed.

Certain reagents affect ciliary movement. Chloroform, ether and carbonic acid inhibit, or temporarily suspend, or even arrest it permanently, if allowed to act for a sufficient length of time. Cold retards and gentle heat accelerates it.

In the respiratory system there are, as has been stated, other cells placed between and beneath the superficial ciliated ones. Those placed between them are long and fusiform, with a nucleus placed in the broad and central part, the cell often extending from the basement membrane to the surface of the epithelium. They are said, when their peripheral end thus reaches the surface, to acquire cilia and take the place of older cells which are being lost. Between them and the basement membrane, are smaller, still younger, nucleated cells, frequently pear-shaped, the stalk projecting upwards and fitting in between the extremities of those above. They correspond with the germinal layer of stratified squamous epithelium.

In the epididymis the cells occur as a single layer, tall and

columnar in shape, with cilia much longer than those of the cells lining the trachea.

In the Fallopian tubes and uterus, the epithelium is more cubical in character, and the cilia are much shorter.

Examine the following specimens :—

(1.) *Gill of mussel in normal saline for ciliary movement.*

Study *ciliary movement* and the *effects of reagents upon it* in the gill of a mussel. It is convenient to use a "cell" in place of a glass slide alone, so that the cilia may not be subject to compression, and also for the purpose of subjecting them to the action of chloroform vapour, etc. Such a cell may be easily made by fixing on a slide a transverse section of glass tubing $\frac{1}{8}$ of an inch or more in depth, and $\frac{1}{2}$ or $\frac{5}{8}$ of an inch in diameter. With the naked eye identify the edge of the gill of a mussel. Excise a portion of it with a pair of sharp pointed scissors, place it upon a cover-glass and invert over the empty cell. With the low power, look round the border of the tissue for a portion which shows a fringe of briskly moving cilia; put on the high power and examine. The movement of the cilia of a given part is constant and in the same direction, but it does not occur at all parts of the surface at the same moment, but rather sweeps across it, as it were, travelling from one point to another. It is this which has caused it to be likened in appearance to a field of corn with the wind passing over it, and bowing it down in successive waves. This can be well seen in the gill of a mussel, especially when the movement has been slowed.

Note the passage of fluid, with any particles it contains, over the surface in the direction of the contraction of the cilia, and also the tendency which the piece of gill has to move out of the field along the surface of the cover-glass, propelled by the action of its own cilia.

Remove the cover-glass, place a small drop of chloroform in the cell with a glass rod; re-adjust the specimen as before, and examine. Observe the gradual slowing down and final arrest of the ciliary movement which, however, re-commences if the application of the vapour is not continued too long. Note, that the contraction of the cilia is more rapid than the subsequent relaxation or straightening.

Remove all traces of the chloroform; if necessary take a fresh piece of the gill, and arrange as before over the empty cell. Place the slide upon the hot stage, and apply heat (*see page 77*).

The movement is quickened until a certain temperature is attained, when heat rigor supervenes. In warm-blooded animals, the maximum temperature is 45°C. , and the minimum 6°C. , at each of which the movement ceases.

(2,) *Isolated ciliated cells from trachea of ox (or pharynx of frog) stained with picro-carmin.* G. J. (Fig. 15.)

Examine with the high power, and note the shape of the cells. Those from the trachea of the ox are elongated and narrow; those from the pharynx of the frog shorter and proportionately broader. In either case observe the bright refractile border at the broad, free end of the cell, the nucleus with two or three nucleoli, the tapering attached extremity, frequently forked, or presenting irregular wing-like projections. In addition, look for small pear-shaped cells from the "germinal" layer and larger fusiform intermediate forms. Goblet or chalice cells are also to be seen with their expanded portion filled with clear mucin, into which the granules of mucinogen have been converted; or the contents of this part of the cell may have been discharged.

(3,) *V.S. Ciliated epithelium of trachea of child, stained with picro-carmin or hæmatoxylin.* G. J. (Fig. 88.)

With the low power, observe the general arrangement of parts: internally, the layer of ciliated epithelium, with its clear refractile border on which the cilia are placed; the whole resting upon a broad homogeneous basement membrane; outside this, the longitudinal layer of elastic fibres, the submucous layer of areolar tissue containing blood-vessels, nerves and lymphatics, and the alveoli of mucous glands, the ducts of which open on to the surface of the epithelium; the ring of cartilage, etc.

Under the high power, study especially the arrangement of the epithelial cells, the cilia, the disc on which they are placed, the position of the nuclei of the different layers of cells, the presence here and there of a goblet cell. Observe the broad, homogeneous membrane on which the epithelium rests. This membrane does not seem to occur in the trachea of most mammals used for histological purposes; at all events not in any degree comparable to that found in man. (See Fig. 15.)

4.—Transitional Epithelium.—This rather unfortunate name is applied to a variety of epithelium which, as far as the number of layers of cells of which it is composed is concerned, may be regarded as "transitional" between simple and stratified squamous

FIG. 13.

A.—COLUMNAR EPITHELIAL CELLS, DISSOCIATED, FROM INTESTINE OF
NEWT, STAINED WITH PICO-CARMINE $\times 450$.

- a.*—Striated border.
- b.*—Nucleus.
- c.*—Nucleolus.
- d.*—Basal processes.

B.—GOBLET CELLS FROM SAME PREPARATION.

- a.*—Nucleus.
- b.*—Remains of protoplasm of cell.



FIG. 14.

A.—ISOLATED CELLS FROM LIVER OF RAT, STAINED WITH PICO-
CARMINE $\times 300$.

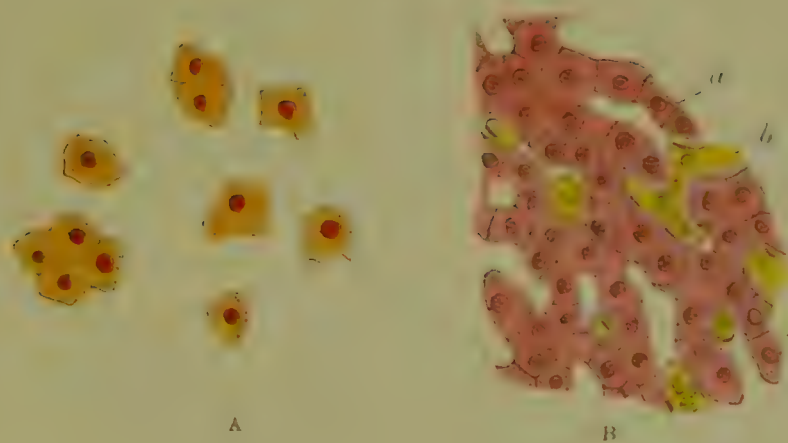
B.—SECTION OF HUMAN LIVER, STAINED WITH HÆMATOXYLIN $\times 300$.

- a.*—Liver cells.
- b.*—Capillaries.

Fig. 13.



Fig. 14



epithelium. It is found lining the pelvis of the kidney, the ureters, the bladder, and a portion of the urethra.

The cells occur in three or four layers. The superficial are large, inclined to be flattened, and are faceted on their under surface in conformity with the convexities of those beneath them. These are large and roughly polygonal in shape, often convex towards the surface, and pointed towards the basement membrane. Beneath these are one or two layers of very much smaller, younger cells, somewhat pear-shaped, the pointed extremity projecting upwards between the cells immediately above, and the broader, flattened end resting on the basement membrane. These correspond with the deeper, or germinal layer of cells elsewhere.

Examine the following specimens :—

(1,) *V.S. Bladder of cat—half-distended—stained with picro-carmin.* F. (Fig. 16.)

Note, with the high power, that the superficial layer of cells is more deeply stained than the rest, and so is easily distinguishable from them. The reason of this is not quite evident. The same deeper staining occurs in the case of hæmatoxylin and other reagents.

Observe the breadth of the cells of the superficial layer. One of these will often be seen to cover the convex surfaces of two, or even three of the cells in the layer immediately beneath, and thus, when isolated, to present several concavities on its under surface.

(2,) *Isolated transitional epithelial cells from bladder of cat, stained in picro-carmin.* G. J.

Under the high power, study the three principal types of cells, the large, superficial, somewhat flattened ones, with their faceted under surface; the intermediate polygonal cells; and the small pear-shaped or cubical cells belonging to the germinal layer. Note the nucleus, stained deeply with the carmin, in the centre of each. Observe how large the nucleus is in comparison with the amount of perinuclear protoplasm in the younger cells. This is equally well seen in the specimen of isolated ciliated cells.

5.—Glandular Epithelium.—Glandular epithelium is found lining the tubules or alveoli of secreting glands, such as the liver, pancreas, and kidney. The classification of the different varieties of glands will be dealt with later, when the structure of those concerned in salivary secretion is described. At present, it will

be sufficient to mention shortly their general plan in construction.

Secreting glands are originally developed from a layer of epithelium, either epiblastic or hypoblastic, by an invagination or down growth of the cells, amongst the connective tissue beneath them. The down growth may be in the form of a simple tube or sac, opening on the surface of the epithelium from which it was derived, and lined with cubical or columnar cells. The glands of Lieberkühn in the intestine are simple tubular glands, and those of the frog's skin, simple sacs. They may be regarded as typical in their structure of glands generally. The differences between them and those more highly developed, are for the most part modifications of arrangement, with a view to increasing the secreting surface at the cost of as little space as possible. The gland is, in its essence, an involuted layer of epithelium, surrounding a lumen communicating with a surface, either directly, or by means of a duct. The gland cells are usually placed upon a *basement membrane*, continuous with that supporting the surface epithelium from which they are derived.

The term *basement membrane* is applied to a class of structures which vary in character in different situations. Sometimes it is homogeneous and apparently structureless, as in the case of the thick translucent membrane supporting the epithelium lining the human trachea. Sometimes it is formed of a layer of flattened connective tissue cells cemented together by their edges to form a complete membrane. In other cases the cells, though flattened, only touch each other by their processes, and a fenestrated arrangement results. In yet other cases the fibres of the connective tissue become flattened and expanded, and take their part in the formation, the intervals between them being occupied by flattened cells.

Starting from these two types—simple tubes and saccules—various degrees of complexity may be attained. The gland may communicate with the surface by a duct, and this latter may have more than one tubule or saccule opening into it. Up to this point it is called a simple tubular or saccular gland, and the duct with its tubules or saccules is called a duct system. When the gland possesses several of these duct systems opening into a larger common one, it is then termed a compound tubular or saccular gland. But, whatever the degree of complexity of a secreting gland may be, its essential characteristics remain the same. It is composed of a layer of secreting epithelial cells placed usually on a basement membrane, surrounded by capillary blood-vessels supported by connective tissue, and bathed with an intervening stream of lymph.

The cells vary very much in shape according to the gland from which they are derived. They are nucleated and may be cubical, columnar or polygonal, and usually occur in a single layer.

Examine the following specimen :—

(1,) *Isolated cells of liver of rat, stained with picro-carmin.*

F. (Fig. 14.)

Under the high power, note the cells scattered over the field—cubical or polygonal in shape, each with a round nucleus, centrally placed, stained deeply with the carmine.

THE CONNECTIVE TISSUES.

Under this heading are included ordinary connective tissue, adipose, adenoid, and mucous tissue, cartilage, dentine, and bone. These belong to the third class of simple tissue, *i.e.*, they are composed of cells placed in a matrix ; they have a passive or mechanical function as contrasted with muscle and nerve ; they are all developed from the mesoblast.

ORDINARY CONNECTIVE TISSUE.

This is found in a loose areolar form, beneath the skin and some mucous membranes, binding together the surfaces which it lies between and at the same time allowing a considerable amount of movement between them. In a denser form, as the fibrous investment of organs such as the lungs, liver, and heart, it serves to protect them from external injury, and sends prolongations into their substance which support their component parts. It affords also a covering to muscles, nerves, and blood-vessels, and penetrates between their ultimate structural elements. Its presence throughout the body is, however, so universal that further enumeration of the localities in which it is found is needless. It occurs in its densest, most inextensible form in tendon, and in its most elastic and extensible in the ligamenta subflava.

For convenience of description the varieties of ordinary connective tissue may be classified as follows :—

Areolar, (*e.g.*, subcutaneous and submucous tissue).

Compact { 1. White fibrous, (*e.g.*, tendon).
 { 2. Yellow elastic, (*e.g.*, ligamenta subflava).

The *areolar*, or loose form of ordinary connective tissue, is the most widely spread tissue of the body. It is found beneath the skin and mucous membranes, and supporting the constituent parts of most of the organs, which it at the same time invests.

FIG. 15.

A.—V.S. EPITHELIUM OF TRACHEA OF CAT, STAINED WITH PICO-CARMINE $\times 300$.

- a.*—Ciliated cells.
- b.*—Goblet or mucin cells.
- c.*—Germinal cells.
- d.*—Basement membrane.

B.—CILATED EPITHELIAL CELLS, DISSOCIATED, FROM PHARYNX OF FROG, STAINED WITH PICO-CARMINE $\times 450$.

- a.*—Ciliated cells.
- b.*—Goblet cell.
- c.*—Cells, germinal, from deeper layer.
- d.*—Cilia.
- e.*—Refractile border.
- f.*—Nucleus.

FIG. 16.

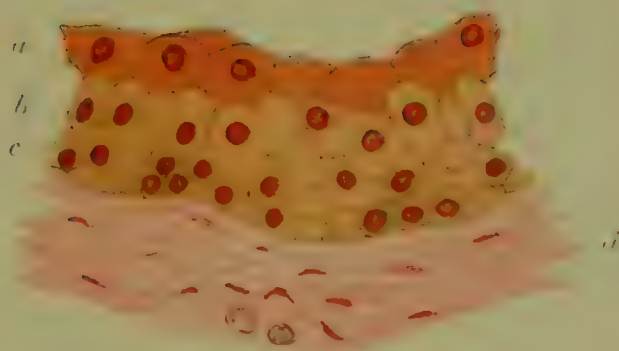
V.S. TRANSITIONAL EPITHELIUM OF BLADDER OF CAT, STAINED WITH PICO-CARMINE $\times 300$.

- a.*—Superficial layer of cells.
- b.*—Middle layer of cells.
- c.*—Deep layer of cells.
- d.*—Fibrous tissue.

Fig. 15.



Fig. 16.



It thus varies a good deal in its density, for whereas where it is subcutaneous it is very loose, and its fibres, white and elastic, form a network with large meshes; where it invests an organ such as the lung, and sends inwards septa for the support of the constituent lobules, blood-vessels, and bronchi, it more nearly approaches the fibrous variety in texture. Again, the omentum may be regarded as one of its looser forms, the fibres being here arranged in the form of a fenestrated membrane, the trabeculæ of which are invested with a layer of flattened epithelial cells.

The *compact* forms of ordinary connective tissue are less widely distributed than the areolar. The fibres usually run in bundles parallel to each other, and are closely united together. In the first subdivision of the compact variety the white fibres predominate over the elastic, and this is found typically in tendons, and also in tough fibrous membranes generally, such as ligaments and aponeuroses, and the fibrous layer of the periosteum of bones; in the second, the elastic are in excess of the white. Yellow elastic tissue in the human body is found in the vocal cords, some of the ligaments of the larynx, and the ligamenta subflava of the spinal column. In many animals, such as the horse and ox, the ligamentum nuchæ is chiefly formed of elastic fibres.

It will be seen from the above, that the fibrous investment of organs, such as the lung, is spoken of as belonging to the areolar variety of connective tissue, and the periosteum of bone to the compact. It is very difficult, however, to draw any definite line between the two subdivisions, as they insensibly merge into each other. The student should again note, that classifications such as these are intended merely for convenience, in teaching and learning the subject.

The Structural Elements of Ordinary Connective Tissue.—These, as already indicated, are cells and fibres, the latter of which are of two kinds, white and yellow, and these elements are embedded in a ground substance or matrix, which is homogeneous, semi-fluid, and nearly allied to mucin. It closely invests the fibres both white and elastic; in the former case penetrating between the individual fibrils, which it cements together. Like the intercellular substance of epithelium, to which it is analogous, it is stained brown with nitrate of silver. The cells occupy well defined spaces in the matrix, which are unstained by this reagent, and thus become clearly revealed.

The spaces vary in shape in the different varieties of the tissue, following closely the outlines of the cells they contain.

The *white fibres* are composed of a number of exceedingly delicate fibrils $\frac{1}{50000}$ to $\frac{1}{20000}$ of an inch in thickness, united together by the cement substance, as the matrix is sometimes termed. The fibres themselves may be a little more or a little less than the breadth of a coloured blood corpuscle, and may run singly, as in the areolar tissue, beneath the skin, or be collected into larger bundles, as in tendon. They are very delicate in appearance, colourless, and faintly striated longitudinally. The individual fibrils never branch; but part of one bundle of fibres may split off to join a neighbouring one. When acetic acid is added, the fibres swell up, become indistinct, and frequently show transverse constrictions, which have been ascribed to the presence of rings of elastic tissue, or to the processes of clasping cells.

The *elastic fibres* are, as a rule, narrower than the white, but they vary in size greatly in different situations and in different animals. In subcutaneous areolar tissue, the thinnest are often not much broader than the constituent fibrils of the white; in the ligamenta subflava they may be $\frac{1}{7500}$ of an inch in diameter, and in the ligamentum nuchæ of some animals, still larger. They are homogeneous, showing no indication of longitudinal fibrillation, and strongly refractile, with a very distinct border. They are pale yellow in colour, and branch and anastomose to form a more or less open network. In a teased preparation of ligamentum nuchæ of ox, the broken ends of the fibres show a rather remarkable tendency to curl upon themselves. The fibres here are very broad, and show in places transverse markings, which may be due to post mortem changes or the action of reagents.

Yellow elastic fibres stain deeply with picric acid and magenta; the white are stained pink by carmine.

The *cells* of ordinary connective tissue vary very much more than the fibres, according to the variety of tissue to which they belong. The fibres, though their arrangement and size are different, always maintain their essential characters. In subcutaneous areolar tissue, four varieties of cells are to be found: (1.) Fixed connective tissue corpuscles—branched or lamellar nucleated masses of protoplasm occupying the cell spaces mentioned above; (2.) Coarsely granular cells, frequently found along

the course of blood-vessels, especially where fat is to be deposited ; (3,) Plasma or vacuolated cells ; (4,) Migratory or wandering amœboid cells. In tendon, on the other hand, the cells are of one kind only, but have very distinctive characters. They are arranged in linear series between the bundles of parallel fibres, and are nucleated and quadrangular, with flattened plates projecting from them. In the *ligamentum nuchæ* of ox the cells are few in number, small, fusiform, lamellar or irregular in shape, and are situated in the white fibrous tissue separating the elastic bundles.

The following forms of ordinary connective tissue will be described, as illustrative of the most noticeable differences in the arrangement and character of the structural elements :—

1.—*Subcutaneous areolar tissue.*

2.—*Tendon and fascia.*

3.—*Ligamentum nuchæ of ox.*

1.—**Subcutaneous Areolar Tissue.**—If a piece of the delicate film of connective tissue, lying between the skin and the subjacent muscles, be gently raised with a pair of forceps, it will be found to the naked eye to consist of a number of interlacing filaments, like “spun glass,” which cross each other in every direction, and in many cases assume a flattened or membranous form. Between these filaments is a system of intercommunicating spaces, or areolæ, occupied by lymph, and from these the tissue derives its name. These spaces must be distinguished from the cell spaces in the filaments themselves, which are only visible under the microscope. In areolar tissue the white and elastic elements are both well represented, but the former predominates. The fibres present, under the microscope, a delicate fibrillated appearance, with a faintly defined outline. They have a wavy course, and cross and interlace with each other in every direction. They are frequently of the breadth of blood corpuscles, but they may be considerably less. The delicate fibrillæ, united together by cement substance to form a bundle or fibre, do not branch. The elastic fibres form a very delicate open network, with much narrower strands, entirely separate from, but intermingled with, the white. The fibres are homogeneous, branching, highly refractile, with a well-defined contour and, though narrower than the white, are broader than the fibrillæ of which the latter are composed. They often pursue a very sinuous course, which is not apparent, however, if the tissue has been stretched. They then appear as

bright refractile lines crossing the field of the microscope in various directions.

The cells of areolar tissue are of four kinds :—

- (1,) Ordinary connective tissue corpuscles.
- (2,) Coarsely granular corpuscles.
- (3,) Plasma cells.
- (4,) Migratory or wandering cells.

The first three are fixed in cell spaces in the mucin matrix, and of these the ordinary connective tissue cell is the most important and most numerous.

(1,) The connective tissue corpuscles are nucleated masses of protoplasm, which vary a good deal in shape. They are often irregularly branched, their processes communicating with those of neighbouring cells. Or they may be lamellar in shape, and applied to the surface of the bundles of fibrils, around which they are folded ; or, if they are placed between three or more bundles, separate lamellæ may be given off to pass between them from the main body of the cell, at this point approaching somewhat to the character of a tendon cell. And it may be noted that the essential cells of the tissue have a remarkable capacity for assuming different forms, according to the necessities of their surroundings, so that they frequently very closely resemble cells of an entirely different type. Thus, where areolar tissue assumes a membranous form, as when its surface becomes condensed to form a basement membrane, or, as in the case of bursæ, to enclose an enlarged lymphatic space, the cells at the surface of the condensation frequently become flattened, and united to each other by their edges to form a more or less complete layer of a distinctly epithelial character ; or they may become flattened, and communicate with each other merely by their processes, the interspaces being occupied by the extended bundles of white fibres between which they lie.

(2,) The coarsely granular cells are found especially where fat is to be developed, (*e.g.*, in the loose tissue around the kidney,) and accompany the smaller blood-vessels. They are oval in shape, nucleated, and contain granules which stain with eosin and methyl-blue.

(3,) The plasma cells differ from the ordinary connective cells, in the presence of numerous vacuoles in their protoplasm. They are nucleated, branched, or fusiform in shape.

(4,) The migratory cells are of the nature of white blood

corpuscles, or lymph cells. They have no essential connection with the tissue, and are merely on their way to or from the blood capillaries or lymph channels.

Arcolar tissue is plentifully supplied with blood-vessels, nerves and lymphatics, but the two former are in the main only passing through on their way to some other structure. The lymphatics are represented by the cell spaces, the areolæ or interstices in the matrix, and lymphatic vessels.

2.—Tendon and Fascia.—A tendon consists of a number of fasciculi of white fibres, closely united together by cement substance to form larger bundles. These run parallel to each other in the long axis of the tendon, and are bound together by an investing sheath of areolar tissue, which sends septa between them from its deeper surface. In this areolar tissue are found blood-vessels, lymphatics, and nerves. The surface of a tendon presents an appearance of alternate light and dark lines, which gives it somewhat the aspect of watered silk. The same thing is noticeable in the case of the larger nerves, and in either case is due to the fact that the longitudinally running fibres are not themselves straight, but pursue a well-marked wavy course. The light falling on the fibres at a different angle in neighbouring parts, is reflected in such a manner as to produce the appearance described.

The tendon bundles are composed for the most part of white fibres, but a few delicate elastic ones are to be found amongst them. The tendon cells are arranged in linear series between the parallel fibres. They are nucleated, somewhat quadrangular in form when seen longitudinally, but stellate in transverse section. This appearance is due to the fact of their being moulded, so to speak, upon the surface of the fibres between which they lie, much in the same way that a piece of wax would be affected by compression between three or more cylindrical lead pencils. The flattened expansions or lamellæ, passing from the body of the cell between the adjacent white fibres, usually unite by means of a fringed border with those of cells in adjacent series. The network thus produced may be beautifully seen in a transverse section of the small tendons of the rat's tail stained with chloride of gold. When the cells are viewed in longitudinal series, as in a teased preparation, the lamellæ are frequently seen projecting towards the observer, when they give the appearance of a dark line running in the long axis of the

FIG. 17.

SUBCUTANEOUS AREOLAR TISSUE OF RABBIT, STAINED WITH
MAGENTA \times 300.

- a.*—White fibres.
- b.*—Yellow elastic fibres.
- c.*—Connective tissue cells.
- c'*.—Connective tissue cells clasping the fibres.
- d.*—Leucocyte.

FIG. 18.

A.—PIGMENT CELLS OF FROG'S SKIN, AFTER EXPOSURE TO
LIGHT \times 250.

B.—MUCOUS CELLS FROM SECTION OF UMBILICAL CORD,
STAINED WITH CARMINE \times 350.

Fig. 17.

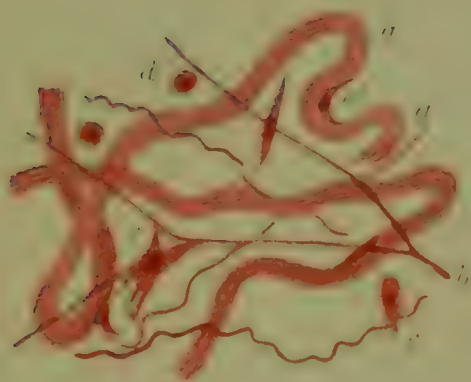
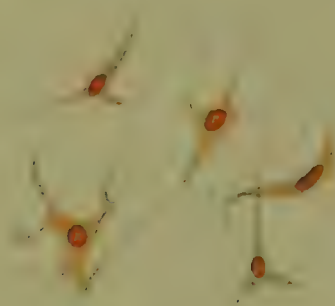
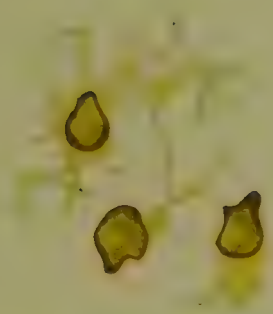


Fig. 18.



cells. These cells are arranged, to a certain extent, in pairs, and the nuclei of each pair are placed at their contiguous ends.

In fasciæ, which are frequently expansions of tendons, the fibres, instead of running all in one direction, frequently cross each other at right angles, but on a different plane; the cells, though still arranged in linear series between parallel bundles of fibres, have not the distinctive characters of typical tendon cells.

In both tendons and fasciæ the vascular supply is small compared with that of muscle, but the capillary network is arranged somewhat in the same way. It consists of branches running longitudinally between the bundles of white fibres, which communicate with each other by transverse loops, and thus form a network with large elongated meshes. The lymphatics of tendons resemble very much those of areolar tissue, except that the cleftlike areolæ are absent. In the central tendon of the diaphragm, stained with nitrate of silver, the system of cell spaces may be particularly well seen, communicating with each other on the one hand, and with the lymphatic spaces which separate the tendon bundles on the other.

3.—Ligamentum Nuchæ of Ox.—This consists of both white and elastic fibres, the latter of which are distinctly in excess. They are large, somewhat triangular or polygonal in transverse section, and are collected into groups or bundles, which run parallel to each other in the long axis of the ligament. The fibres anastomose freely with each other, so as to form a network with elongated narrow meshes. The white fibrous element invests the whole ligament, and penetrates between the groups of elastic fibres, and surrounds the fibres themselves. It is somewhat areolar in nature, resembling the supporting connective tissue sent in from the fibrous investment of many organs. The cells are small and somewhat flattened, and apparently are not associated with the elastic fibres, but belong to the areolar tissue.

Blood and lymph channels are found in small quantities in the areolar tissue supporting the elastic network.

DEVELOPMENT OF CONNECTIVE TISSUE.

The mesoblastic cells of the embryo, at first round, become branched, and anastomose with each other. The lymph which bathed them assumes the characters of mucin, and represents

the matrix or interstitial cement substance. In this matrix two kinds of fibres, white and elastic, are developed, and of the nature of this process two views have been held:—

(1.) *That the fibres are produced by a transformation of the protoplasm of the cells.* In the case of the white fibres it is supposed that successive layers of cell protoplasm become converted to fibrillar tissue, the cell remaining of its original size in virtue of compensatory growth. In support of this view the cells are frequently seen in an elongated condition, with their extremities much drawn out and fibrillated; and it is also common in young connective tissue to observe the cells, somewhat oat-shaped when seen laterally, closely applied to one side of a white fibre, in process of development, of which they seem to form a part. The appearance, in fact, is such as one would expect to find if the white fibre was being in some way produced from the cells lying upon it. This was the view entertained by Beale and others, who regarded the body as composed of two classes of structures—"formed" and "formative"—the latter being represented by protoplasm, and the former by the fixed, inactive material to which it was supposed to have given rise or been converted. The elastic network, according to this theory, is produced by a transformation of the anastomosing processes of some of the cells.

This theory is often favoured very much by the appearances to be observed, but, as will be seen, there are considerable difficulties in the way of its general acceptance.

(2.) *That the fibres, white and elastic, are produced by a deposition of material in the intercellular matrix, possibly under the influence of the cells, but unassociated with any transformation of their protoplasm.* In favour of this view may be quoted the appearance of the elastic network in the arytenoid cartilages of the ox or sheep. Here we have at one part hyaline, and at another yellow elastic cartilage. On passing from the purely hyaline area towards the elastic, the fibres of the latter are seen to appear in the clear matrix entirely apart from the cells, becoming more and more plentiful as the purely elastic portion is approached. The picture is exceedingly striking, and can leave no doubt in the mind that in this case the cells take no part, as far as a change in their protoplasm is concerned, in the formation of the elastic network. Again, in embryonic mucous tissue, which is becoming transformed to ordinary con-

nective tissue, the appearances are equally conclusive. Here we have a matrix of mucin in which are scattered branched connective tissue cells, and when the development referred to takes place, fibres, both white and elastic, appear in the matrix entirely apart from the cellular elements.

That the fibres are formed by a transformation of the cell protoplasm seems improbable, in the face of what we know of the manner of production of other forms of connective tissue. The fibrillated lamellæ forming the organic basis of the intercellular part of bone, is undoubtedly produced under the influence of the osteoblasts, by a deposition of material around them, and not by a transformation of their cell substance. The fibrous basis of the intercellular part of hyaline cartilage, which is usually accepted as resulting from the fusion of successive cartilage capsules, cannot be regarded as due to a conversion of part of the cells themselves. To take yet another instance, that of the development of the dentine of the teeth ; there is again no evidence of a process of transformation of the odontoblasts themselves, although the dentine is very evidently produced under their influence. In each of these three cases we have an organic structure with strong analogies to the fibres of ordinary connective tissue, created in the immediate neighbourhood of the cells, and, it may be granted, under their influence, but in no way in structural continuity with them.

In the case of the appearance of granules or fibres of elastin in the arytenoid cartilage, it is to be noted that this takes place first in the neighbourhood of the cells, although there is no connection between them ; and it may perhaps provisionally be supposed that both the white and elastic fibres of ordinary connective tissue are produced by the influence of the cells between which they appear ; but, the nature of that influence is not clear. In the case of bone, hyaline cartilage, and dentine, where the cells are in immediate contact with the structural basis produced, the process has some resemblance to that of secretion, and this is also the case in ordinary connective tissue when the cells are closely applied to the fibres ; when the fibrous element appears in the matrix distinctly apart from the cells, the analogy to secretion is by no means so obvious.

Examine the following specimens :—

(1.) *Subcutaneous areolar tissue, fresh preparation, unstained.*

Remove the skin from the inner side of the thigh of a rat or

FIG. 19.

T.S. TENDONS OF RAT'S TAIL, STAINED WITH GOLD CHLORIDE $\times 350$.

- a.*—Fibrous tissue sheath of tendons.
- b.*—Tendon bundles.
- c.*—Tendon cells.

FIG. 20.

T.S. TENDON (HUMAN), STAINED WITH HÆMATOXYLIN $\times 50$.

- a.*—Fibrous investment of tendon.
- b.*—Septa from investment of tendon.
- c.*—T.S. Tendinous bundles.
- d.*—Tendon cells.
- e.*—Artery and vein in tendon sheath.

Fig. 19.

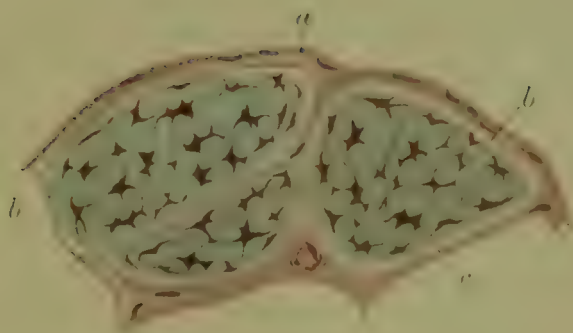
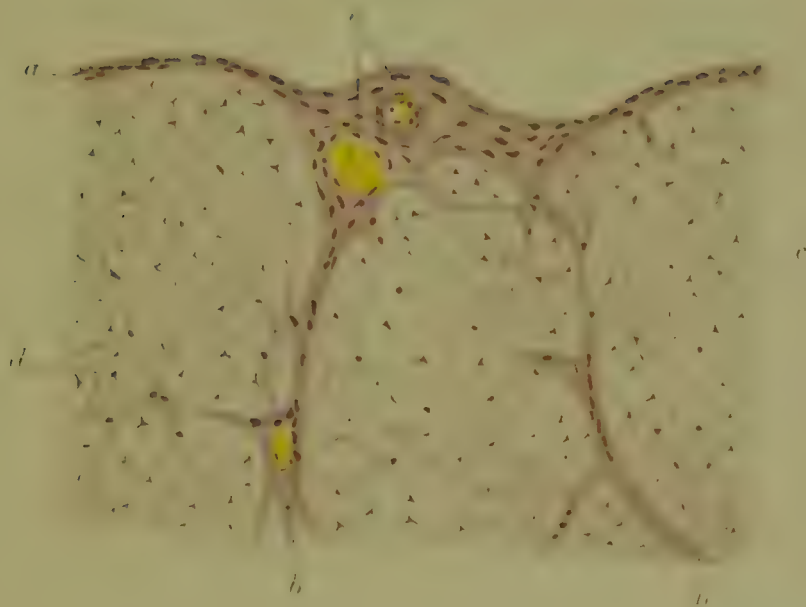
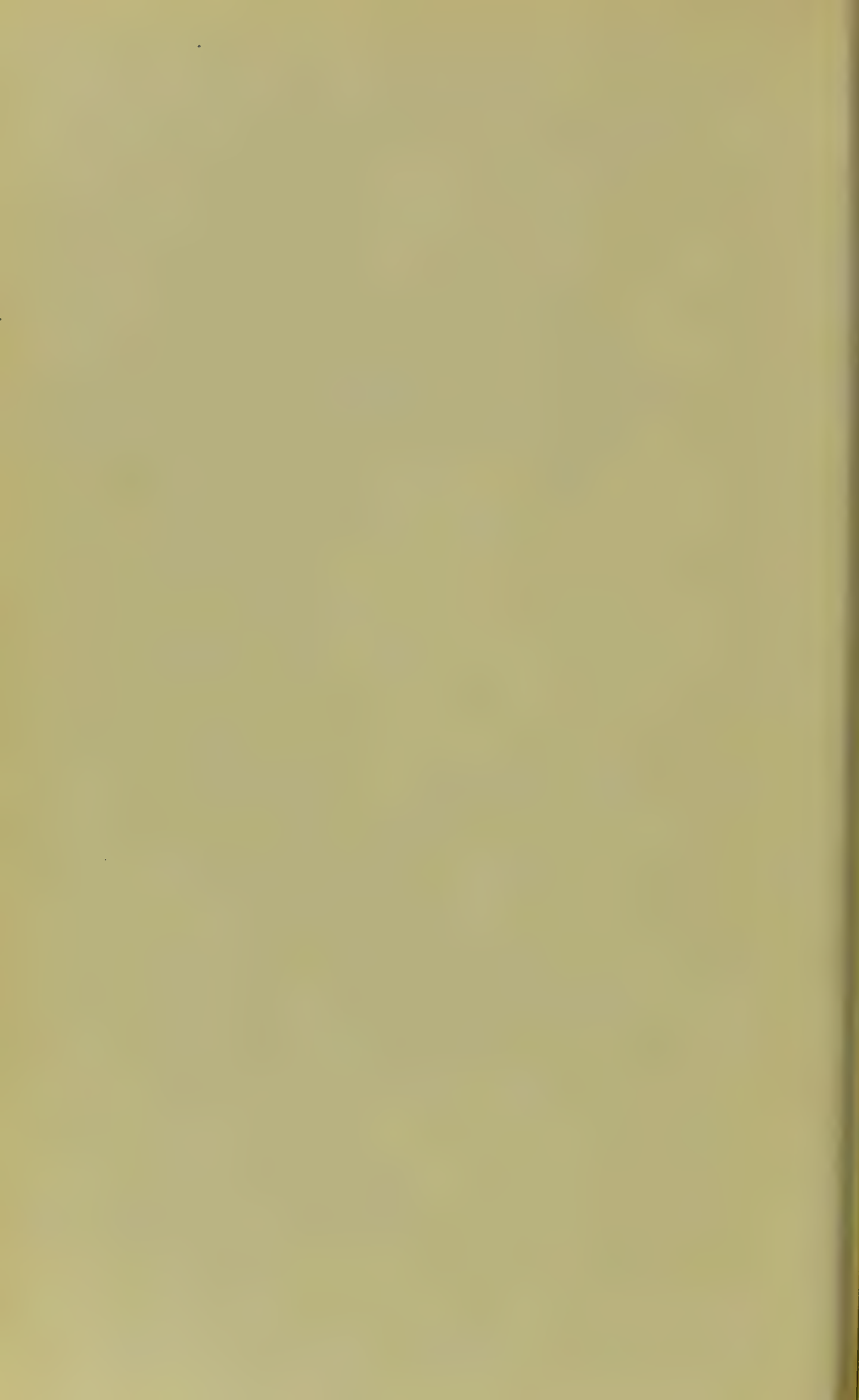


Fig. 20.





rabbit, dissect off a small piece of the loose tissue lying on the muscles, and place it on a slide. Stretch it out with needles, so as to have as thin a layer as possible, add a small drop of normal saline, and cover. When the tissue is first removed from the body, it runs up into a small white mass upon the forceps, and a little care is required to get it successfully spread upon the slide, to which it closely adheres. It is advantageous to breathe upon it while doing this, in order that it may not become dry.

With the high power, look first at the wavy white fibres: they are easily distinguishable from the elastic by their breadth, their faint outline, and fibrillated appearance. Observe that the breadth varies, some being a good deal less than the broad diameter of a red blood corpuscle. Note the intricate interlacement they form with each other, although the fibrils do not themselves branch as the elastic fibres do. At the same time a fibre, or part of one, may leave one bundle to become associated with another.

The elastic fibres stand out very distinctly in virtue of their great refractile power. Observe the large open network of narrow unfibrillated strands with a distinct contour. They frequently pursue a sinuous, almost corkscrew-like course, but this is often concealed by the stretching they have undergone. They then appear as perfectly straight, bright, ruled lines, often joining each other at an angle: but this is not their natural aspect.

Look for the corpuscles, especially the ordinary connective tissue cells—branched, lamellar, or fusiform. They are by no means easily seen without the help of a reagent, as their protoplasm is exceedingly delicate. Irrigate with a 1 per cent. solution of acetic acid, which brings the nuclei of the cells clearly into view. Many of the nuclei are fusiform in shape, and appear closely applied to the white fibres. Surrounding them, a little delicate, finely granular protoplasm will, in some cases, be made out with a little care. Observe also, the triple nuclei of the small leucocytes or migratory cells. The other two varieties, granular and vacuolated, are not so easily seen in this specimen. Now look at the white fibres, which have become swollen up and still more indistinct, while the elastic are more clearly revealed in consequence. The former sometimes show a peculiar series of annular constrictions, as if the bundles had been, previous to their swelling, surrounded with rings at inter-

vals, which now cause constrictions. In some cases, instead of separate rings, the constriction appears in the form of a continuous spiral round the fibre. The constricting band has been supposed to be elastic in its nature, as it is unaffected by the acetic acid; in some cases it may be due to the processes of the clasping cells passing entirely round the fibre.

(2.) *Stain a fresh preparation with magenta*, and examine in glycerine or Farrant's solution. (Fig. 17.)

Note, that the cells and fibres are stained red with the reagent, the nuclei and the elastic fibres being especially revealed.

(3.) *Subcutaneous areolar tissue, stained with nitrate of silver.*

Spread a piece of areolar tissue upon a slide, and cover it with a large drop of $\frac{1}{4}$ per cent. solution of nitrate of silver. Allow to remain for ten to twenty minutes; remove with bibulous paper, add a drop of glycerine, or Farrant, and cover. Reduction in this case takes place after mounting, when the specimen must be exposed to light for several hours. Under the high power, observe that the general ground substance is stained of a deep brown colour, against which the unstained cell spaces stand out very clearly. Note their branching, irregular shape, and that they anastomose one with another, to form a system of communicating lymph channels throughout the tissue.

As nitrate of silver is a hardening reagent, the specimen is a permanent one. The student, however, may require to make more than one before he attains a successful result.

(4.) *Tendon of rat's tail, stained with chloride of gold, teased, and mounted in Farrant's solution.* (Fig. 22.)

Examine first with a low power, and observe the deeply stained tendon cells arranged in linear series, between the more lightly stained bundles of fibres. Select a portion in which the cells are seen clearly, and put on the high power. Study the tendon cells; they are rectangular, oblong, placed end to end. The nuclei of two adjacent cells are placed in their adjoining ends; they are unstained by the chloride of gold, and look almost white against the deeply stained protoplasm of the rest of the cell; and as they are large, and often entirely fill the end of the cell they belong to, they are liable to be overlooked, a student supposing the cell to end where the nucleus begins. On more careful examination, each cell may be seen to give off from its sides, flattened plates, which extend a variable distance,

and break up into a fringe of very delicate processes, which anastomose with those from cells in adjacent rows. There is usually no difficulty in finding some part of the preparation which will show these lamellæ and processes. A tendon cell gives off four or five of these plates from its circumference, but looked at longitudinally, those given off either before or behind the cell, appear as lines running longitudinally upon it. It is this line which is sometimes called Boll's stripe. Such a line on any cell appears continuous or interrupted according to whether the lamella is itself in focus, or the fringe of processes springing from it; in the latter case, of course, it is interrupted.

(5.) *T.S. Tail of rat, stained with gold chloride, (for T.S. of tendon cells). B. (Fig. 19.)*

Examine first with the low power. Observe the round or oval transverse sections of the tendons of the tail surrounding the bone of the vertebra, or the cartilage of the intervertebral disc. Select one of the tendons and examine it with a high power. Observe the tendon cells, stained deeply with the reagent; in transverse section they have a stellate appearance, due to the lamellæ, springing from them, being cut across. Note how the lamellæ of one cell join with those of others to form a very complete network, the meshes of which are occupied by bundles of white fibrous tissue, which run longitudinally, and are seen therefore in transverse section. Thus, each tendon cell or row of cells is surrounded by cylinders of fibrous tissue, and the shape of the cells is in conformity with the space left between these cylinders.

(6.) *Tendon of rat's tail, stained with hæmatoxylin, teased (for fibrillæ of white fibres). F.*

The piece of tendon has been previously stained in bulk in hæmatoxylin. Fix one end on the slide with one needle, and with the other, fray out the opposite extremity. A better result is usually obtained thus than by attempting to tease the whole piece. Put on a drop of Farrant's solution, and cover.

Observe the delicate ultimate fibrillæ. The tendon cells are not well seen in this specimen.

(7.) *Tendon of rat's tail, stained with nitrate of silver, mounted in balsam (for cell spaces). (Fig. 21.)*

In mounting this specimen, particular care should be taken in carrying out the processes of dehydration and clarification. They are best done in watch glasses, and more time is required

FIG. 21.

TENDON OF RAT'S TAIL $\times 300$.

A.—Stained with gold chloride.

B.—Stained with silver nitrate.

FIG. 22.

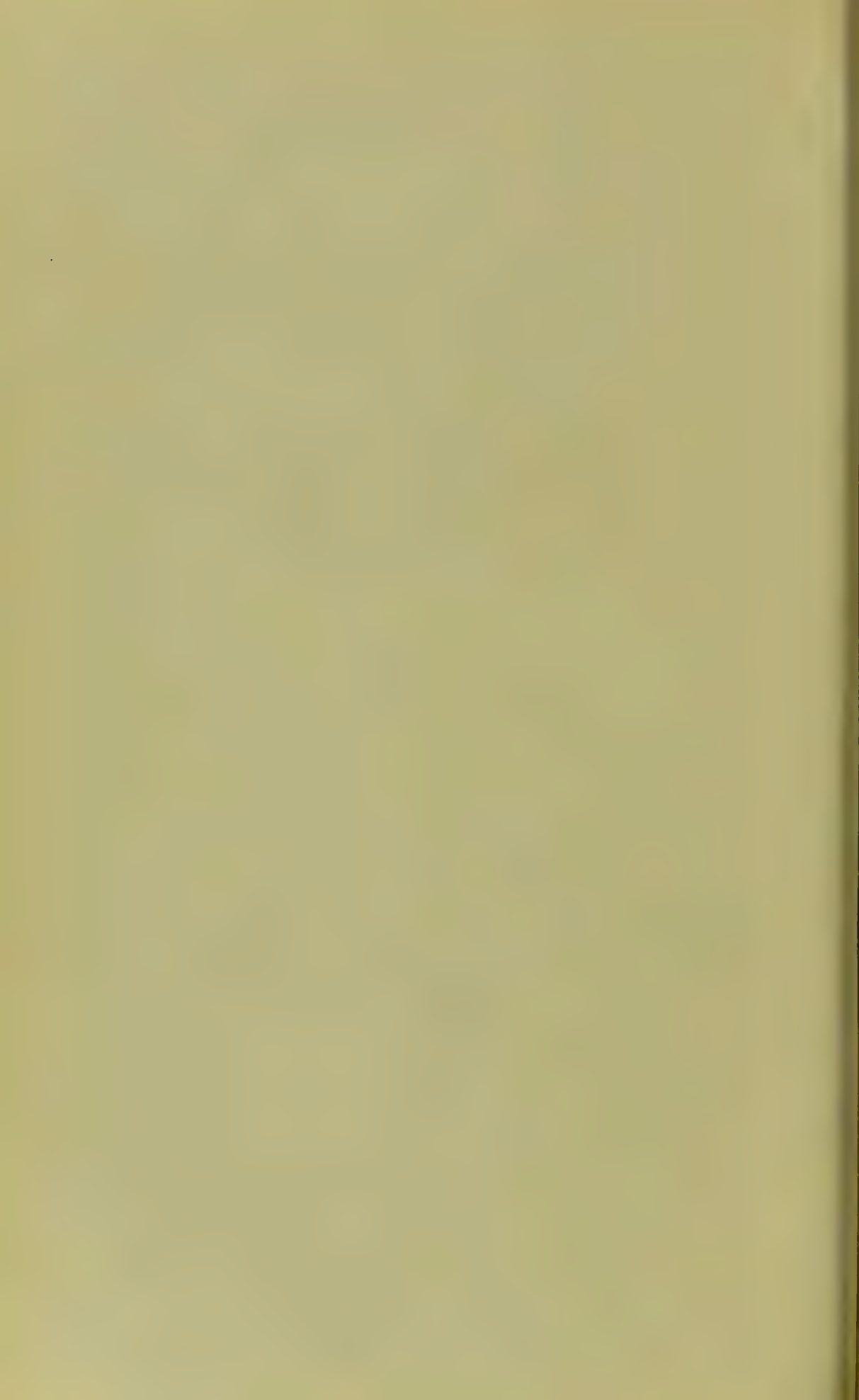
A.—TENDON CELLS OF RAT'S TAIL AS SEEN IN SITU, STAINED WITH GOLD CHLORIDE $\times 250$.*a.*—Nuclei—unstained.*b.*—Boll's line.B.—TENDON CELLS OF RAT'S TAIL, STAINED WITH GOLD CHLORIDE $\times 500$.*c.*—Flanges.*d.*—Processes.*e.*—Boll's line.C.—TENDON CELL FROM RAT'S TAIL, STAINED WITH GOLD CHLORIDE $\times 1000$. (SEMI-DIAGRAMMATIC).*c.*—Flanges.*d.*—Processes.*e.*—Boll's line.

Fig. 21.



Fig. 22.





than in dealing with a section. The specimen must not be teased.

Under the high power, notice that we have here an exact reversal of the staining with chloride of gold; in fact, the one is the "negative" of the other. In the case of chloride of gold, it is the cells which are stained, to the comparative neglect of the interstitial material. Here, the latter reduces the silver nitrate, and is stained brown, the cells being unaffected, and, consequently the spaces in which they lie are very distinctly revealed. Observe the shape of the cell spaces, corresponding exactly with that of the cells, as seen in the chloride of gold preparation; their arrangement in linear series, the communications between those in the same and adjacent rows, etc. In some part of the preparation, the polygonal outlines of the epithelial cells, covering the tendon, may be visible; the cement substance between them being stained brown with the silver nitrate.

(8,) *T.S. Human tendon, stained with hæmatoxylin. B. (Fig. 20.)*

In such a specimen, study first under the low, and then with the high power, the general arrangement of the parts. Observe the investment of areolar tissue (more dense than when subcutaneous) sending in septa from its deeper surface, dividing and subdividing the fibrous tissue of the tendon into larger and smaller fasciculi. Recognise the blood-vessels, nerves and lymphatics, in both the sheath and the septa given off from it. With the high power, note the stellate tendon cells, in the transversely cut fibrous tissue of the tendon, bounded by the septa. They are not, however, nearly so distinctly seen in a specimen stained in this way as in the T.S. gold chloride one. Look for transverse sections of elastic fibres here and there amongst the white; they are seen as bright refractile points.

The fasciculi of a tendon send off slips which run longitudinally to join neighbouring fasciculi, involving a commensurate change in the disposition of the septa bounding them; so that transverse sections of the same tendon are not necessarily replicas of each other as far as the outlines of the fasciculi are concerned, even when taken from the same neighbourhood; unless, of course, they are next in series, or nearly so.

(9,) *Ligamentum Nuchæ of ox, semi-digested, teased in picric acid. F. (Fig. 23.)*

Observe the homogeneous, highly refractile, anastomosing fibres which tend to curl up at their broken ends. They are very

much broader than those of areolar tissue. They show in some places transverse markings, which are probably due to a *post mortem* change, or the action of reagents. The white fibrous element in this specimen has been dissolved in the process of digestion.

(10,) L.S. *Ligamentum Nuchæ of ox, stained with picro-carminé. F.*

Under the high power, note the elastic fibres, stained yellow with the picric acid, running longitudinally, and giving off branches to anastomose with each other; and the white fibrous tissue between them, stained pink with the carmine. Note the excess of the elastic over the white fibrous tissue.

(11,) T.S. *Ligamentum Nuchæ of ox, stained with picro-carminé. F.*

With the high power, observe the yellow elastic fibres, cut transversely, and arranged in groups; and the carmine stained, white fibrous tissue, surrounding both the groups and the fibres individually. The sections of the latter are not perfectly round, but possess one or more angles usually directed towards the centre of the group.

APPENDIX TO CHAPTER IV.

METHODS OF PREPARATION.

1. **Newt's Moul.**—If a frog or newt be kept for a few days in a little water, the superficial layer of the epidermis separates, in the form of a delicate membrane. Harden for a day in methylated spirit; stain in weak hæmatoxylin for twenty-four hours; cut up with scissors into appropriate pieces; mount in either Farrant or balsam.

2. **Omentum of cat.**—Stain with nitrate of silver and hæmatoxylin; mount in Farrant or balsam. (*See page 45.*)

3. **Small Intestine of rabbit** (muscular wall), injected with nitrate of silver.

Kill a rabbit, expose the heart, and cut off its apex. Through the left ventricle pass a glass cannula into the aorta, and tie it firmly in position with thread, passed round the vessel with an aneurism needle. Fill a glass hand-syringe with distilled water, connect it with the cannula, and slowly force the fluid through the systemic arteries, capillaries and veins, back to the right side of the heart, where it issues, at first mixed with blood, from the cut end of the right ventricle. Pass three or more syringefulls of distilled water through the body in this way, till the fluid issuing from the right ventricle is quite clear. As the blood becomes washed away, the tissues become paler in colour and œdematous. This is particularly marked in the case of the tongue. Now substitute a $\frac{1}{4}$ per cent. solution of nitrate of silver for the distilled water, and continue forcing it through the vessels for about ten minutes or a little longer. When it is considered that the epithelial lining of the vessels has been subjected to the solution for a sufficient length of time, again inject distilled water to wash out all the silver solution from the lumen of the tubes. It will be remembered, that in staining the omentum with this reagent, it was washed, both before and after subjection to it, in distilled water, and it is the same principle which is followed here. If the silver solution is not thus washed out, it tends to become precipitated in the vessels, and the preparation may in this way be completely spoiled. All that is wanted is that the interstitial cement substance between the epithelial cells lining the vessels shall become impregnated with the silver salt.

Now remove the syringe, open the abdomen, excise a portion of the small intestine. Slit it up, and lave in distilled water till quite clean. Then expose to light in a porcelain vessel in 50 per cent. solution of spirit. As reduction takes place, the course of the blood-vessels of the muscular wall of the intestine become beautifully traced in brown beneath the thin peritoneal

covering. Transfer to methylated spirit for preservation. When a specimen is to be mounted, select a portion, separate it with scissors, and lay it upon a slide with the rough, mucous internal surface uppermost. Fix the left end with the finger or otherwise, and with a scalpel scrape gently away the mucosa from the rest of the specimen, leaving only the thinner muscular coat, with the blood vessels traced distinctly in it. Dehydrate and clarify in watch glasses ; mount in balsam.

When successful, the preparations are very beautiful. A frog will do as well as a rabbit, and the consequences of failure are not so serious ; but the number of specimens obtainable is, of course, much fewer.

4. **V.S. Hard Palate of cat.**—Harden in Müller's fluid ; cut in gum ; stain in picro-carminé ; mount in Farrant. A little care is required in dissecting the membrane from the bone beneath it, to which it is very firmly adherent.

5. **V.S. Small Intestine of cat.**—Remove a small part of the intestine from a cat directly after death ; cut into pieces, wash rapidly in water, and place in an excess of Müller's fluid. Change the fluid frequently, adding spirit after the first ten days. Cut in gum, stain in hæmatoxylin, and mount in either Farrant's solution or balsam, according to the translucency required ; or pieces of the tissue may be stained in bulk in borax-carminé, and cut in paraffin.

6. **Isolated Cells from intestine of newt.**—Place the intestine of a newt, slit up, in $\frac{1}{3}$ alcohol for twenty-four hours, then in $\frac{1}{2}$ per cent. osmic acid for six hours, and stain in picro-carminé for twenty-four hours. Scrape the mucous surface, and diffuse the scraping in glycerine jelly. (*See page 4.*)

7. **Isolated Cells from the trachea of ox or frog.**—Treat in the same way as 6.

8. **Isolated Cells from bladder of cat.**—Treat in the same way as 6 and 7.

9. **Isolated Gland Cells of liver of rat.**—Place small cubes $\frac{1}{8}$ in. diameter, in $\frac{1}{2}$ per cent. osmic acid for twenty-four hours, and transfer to picro-carminé for a similar period. Detach a small portion of the peripheral layer, and break up with needles on a slide ; add a drop of Farrant's solution, and cover.

10. **V.S. Epithelium of bladder of cat.**—Harden in 2 per cent. solution of ammonium bichromate, cut in gum, stain in picro-carminé, and mount in Farrant ; or stain in bulk in borax-carminé, cut in paraffin, and mount in balsam.

11. **V.S. Epithelium of trachea.**—Harden the trachea of a child in $\frac{1}{4}$ per cent. solution of chromic acid ; cut in gum, stain in hæmatoxylin, and mount in Farrant or balsam.

12. **Tendon of rat's tail, stained with gold chloride, teased.** (*See page 48.*)

13. **T.S. Tendon of rat's tail stained with gold chloride.**—Stain a small portion of a rat's tail, from which the skin has been removed, by the boiled gold chloride method. (*See page 48.*) Harden in alcohol, decalcify with chromic and nitric fluid ; cut in gum, or preferably paraffin ; mount in balsam. When cut in gum the parts tend to separate from each other.

14. **Tendon of rat's tail, stained with hæmatoxylin, teased.**—Place the

fresh tendon of a rat's tail for twelve hours in 1 per cent. osmic acid solution, and stain for twenty-four in weak hæmatoxylin. Tease, and mount in Farrant.

15. **Tendon of rat's tail, stained with nitrate of silver.** (*See page 46.*)

16. **T.S. Human Tendon, stained with hæmatoxylin.**—Harden the tendon in Müller's fluid, without spirit, as it tends to become very hard; cut in gum if possible; stain; mount in Farrant or balsam. The sections, however, tend to fall to pieces, and it would be highly advantageous to stain in bulk in borax-carmines and cut in paraffin, were it not that the tendon by this method is made still harder, and usually refuses to cut. If it is attempted, the tendon should be distinctly under-hardened rather than the reverse, before the process is commenced.

17. **Ligamentum Nuchæ of ox, semi-digested, teased.**—Digest portions of the lig. nuch. of ox $\frac{1}{8}$ in. in diameter, in either gastric or pancreatic fluid, till they appreciably soften in consistence, and fragments can readily be separated by tearing them away with a pair of forceps. Tease a small piece in picric acid solution, or water; add Farrant; cover.

18. **L.S. and T.S. Ligamentum Nuchæ of ox.**—Harden in Müller's fluid, cut in gum, stain in picro-carmines, and mount in Farrant or balsam. A good method to reveal the nuclei of the white fibrous tissue, is to stain small pieces in borax-carmines in bulk, and cut them in paraffin.

CHAPTER V.

*THE SIMPLE TISSUES (Continued).**ADIPOSE, ADENOID, AND MUCOUS TISSUES;
PIGMENT CELLS; AND CARTILAGE.***ADIPOSE TISSUE.**

THIS is a modification of areolar tissue, in which the connective tissue cell is distended with a globule of oil, into which its protoplasm has been converted. It is found following the distribution of areolar tissue in many parts of the body: in the cutis vera of the skin; surrounding the kidneys; filling up the furrows on the surface of the heart, and sometimes enveloping the organ to a greater or less extent; in the omentum; filling the depressions in the neighbourhood of joints, etc. A fat cell is usually round or oval in shape, and possesses a cell envelope enclosing a globule of fat. The nucleus of the cell from which it was developed remains in a flattened form, surrounded by a small amount of the original protoplasm at one side of the cell between the oil globule and the cell wall. The rest of the protoplasm has usually entirely disappeared, but sometimes a very thin layer remains beneath the envelope. When the fat cells have been produced in sufficient numbers to cause mutual compression, their contour is frequently polygonal with rounded corners. Their development may be conveniently studied in a vertical section of the foetal scalp, stained with osmic acid. The cutis vera here consists of growing areolar tissue, and fat cells may be seen in the deeper parts of it in every stage of formation. First, a very small droplet, or more than one, appears in the perinuclear protoplasm of the young connective tissue cell, and, as the drops increase in number at the expense of the protoplasm, they tend to run together to form larger ones. When the process is complete, all, or nearly all of the protoplasm has

become converted, and the now distended cell consists of one large globule of fat or oil, surrounded by the original cell wall, the flattened nucleus (with a little of the protoplasm around it) intervening at one side. The cell has then rather the appearance of a signet ring, the nucleus representing the signet, the protoplasm the part of the ring in which it is set, and the remainder of the cell wall the hoop. The fat cells, with a little delicate connective tissue between them, supporting a capillary network, are collected into lobules, and these again into still larger ones or lobes. The tissue is freely supplied with blood. A small artery passes to each lobule, breaks up into capillaries, which ramify between the individual cells, and unite to form one or two small efferent veins.

The fat globules are stained black with osmic acid, but are dissolved by the action of alcohol. Crystals of margarine sometimes appear in fat cells. They may be produced artificially by keeping a small portion of fat in glycerine for a day or two.

ADENOID TISSUE.

This is another modification of areolar tissue. It is found forming the basis of lymphatic glands; in the tonsils, thymus gland, and spleen; in the mucosa and sub-mucosa of the alimentary tract, where it forms solitary glands and Peyer's patches; and in other situations.

It consists of an exceedingly delicate network of fine fibrils, of the nature of white fibrous tissue, covered with branched nucleated cells. The body of the cell containing the nucleus occurs at the node, and the branches are applied to the fibrillar strands passing from it. Thus, unless the cells are artificially removed from the network they cover, the fibrillar basis is not seen, and the tissue appears to consist of branching, anastomosing, nucleated cells. The fibrils correspond with the white fibrils of areolar tissue, and the cells to the fixed connective tissue corpuscles; the intercellular mucin-like matrix has disappeared, and the meshes of the network are occupied by lymph. But in addition to the lymph, there are to be found in many situations, numerous lymph corpuscles, cells resembling the white corpuscles of blood, but with relatively little perinuclear protoplasm, and a single large nucleus. These may be so closely packed as entirely to obscure the fibrillar reticulum covered with branching cells, as in the

FIG. 23.

YELLOW ELASTIC TISSUE, TEASED, FROM LIGAMENTUM NUCHÆ OF OX
STAINED WITH PICRIC ACID $\times 300$.

a.—Fibre showing point of division.

b.—Transverse markings.

FIG. 24.

A.—FAT CELLS FROM SECTION OF CUTIS VERA OF SKIN, STAINED WITH
OSMIC ACID AND Picro-CARMINE $\times 250$.

a.—Fat cell showing remains of protoplasm in which is seen the nucleus.

b.—Fat cell showing crystal of margarine.

c.—Connective tissue.

B.—DEVELOPING FAT CELLS FROM SECTION OF CUTIS VERA OF FETAL
SKIN, STAINED WITH OSMIC ACID $\times 300$.

a.—Fully developed fat cell.

b.—Fat cells in earlier stages of development.

c.—Connective tissue cells.

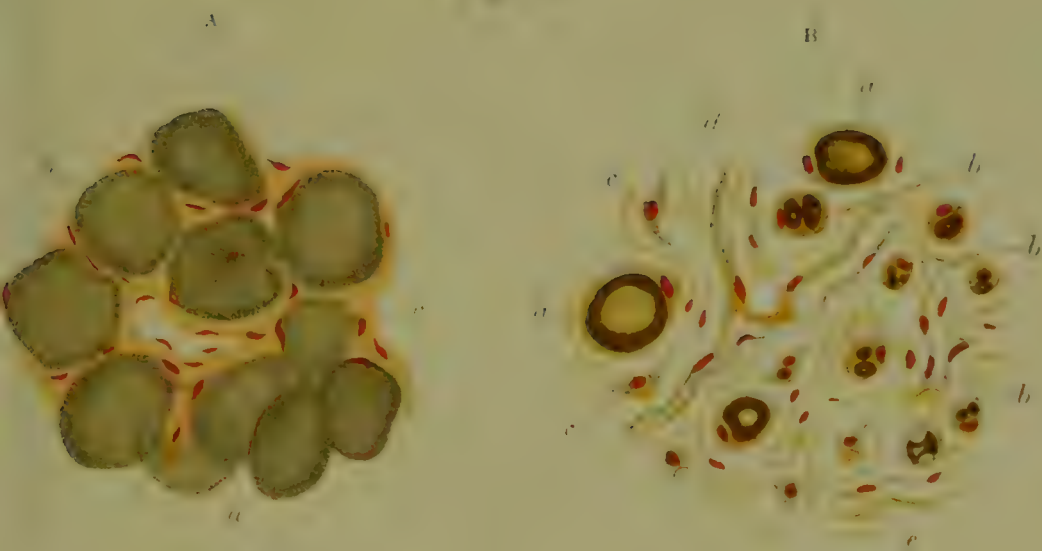
d.—Developing capillary blood-vessel.

e.—Connective tissue fibre.

Fig. 23.



Fig. 24.



follicular portion of a lymph gland, and in the solitary follicles of the alimentary canal. On the other hand, in the sinus of a lymph gland they are much less numerous, and the network, which is here coarser than elsewhere, can very readily be seen. In the spleen, the meshes of the network are, to a large extent, filled with blood corpuscles.

MUCOUS TISSUE.

Mucous tissue is for the most part an embryonic structure. In the embryo it is found beneath the skin, and it also forms the jelly of the umbilical cord. In the adult it is found as the vitreous humour of the eye. It consists of a matrix of mucin, in which are embedded nucleated branching cells, with long anastomosing processes. In the umbilical cord at birth, however, numerous fibres have already begun to appear in the intercellular substance, and the tissue is not at this period of development typically mucous; it is in process of transformation to ordinary connective tissue.

PIGMENT CELLS.

In the human subject pigment cells of the connective tissue type are found in great numbers in the choroid coat of the eye, and in the iris. In many animals they are much more common, and are very readily studied in the skin of the web of the frog's foot.

In the choroid they are large, somewhat flattened branched cells, the protoplasm of which is laden with numerous granules of a black or brown pigment, melanin. The nucleus is not pigmented, and is sometimes very beautifully seen in contrast with the surrounding pigment in stained preparations, if the cells are seen on the flat. In some cases it is, however, entirely obscured by the pigmented perinuclear protoplasm. In the frog's skin the pigment cells present different appearances according to their reaction to light. If the frog has been exposed to daylight the pigment is collected in the centre of the cell, leaving the branches clear. If, on the other hand, it has been kept in the dark, the pigment is evenly diffused. The cells are thus under the influence of the nervous system, the afferent nerve being the optic.

Epithelial cells also contain pigment in some situations, as in the case of the hexagonal pigment cells of the retina, and the cells of the deeper part of the stratum Malpighii in the skin of the negro.

Examine the following specimens :—

(1,) *V.S. Human skin, stained with osmic acid and picro-car-mine. F. (Fig. 24 A.)*

Under the low power identify the clusters of large cells. in the deeper part of the cutis vera, stained brown or black with the osmic acid. Select a group, put on the high power, and examine. The structures shown in the figure are easily made out, namely, the somewhat oval or round fat globules stained with the osmic acid, the envelope of the cells surrounding them, with the carmine-stained flattened nuclei causing a projection at one point. It is not usually easy with a power of 250 or 300 to observe any protoplasm surrounding the nucleus in a fully developed fat cell, as it is very small in amount, even when present. The remains of the protoplasm are, however, very easily seen in the next preparation, in which the cells have not yet reached maturity. Notice the transparency of the fat cells. Even in a specimen stained with osmic acid the outlines of those in a deeper plane, when there are several layers, can be seen through the superimposed cells (*a*).

(2,) *V.S. Fœtal scalp, stained with osmic acid and picro-car-mine. F. (Fig. 24 B.)*

As before, under the low power, identify the clusters of fat globules, distinguished by their staining with osmic acid. Even under this power, observe the very varying sizes of the globules making up a cluster. Those at the periphery are, as a rule, the smaller, as these are developing clusters, and the cells are first formed from connective tissue corpuscles in the centre, the patch or cluster growing by the continuation of the process at its periphery. Hence are here to be found the more recently forming fat cells—connective tissue cells with one or more small fat globules inside them.

Put on the high power, and investigate a portion of a cluster showing cells in process of transformation. Find a young connective tissue cell (*b*), containing in the first place only one or two small particles of fat or oil (stained black or brown with the reagent), and then follow the subsequent stages through different cells until the fully developed fat cell is reached (*a*). Observe the

shapes of the young connective tissue corpuscles in which the process takes place. They are not large and elaborately branched as in adult areolar tissue, but are comparatively small, round, oval, oat-shaped, or pear-shaped, with a rounded nucleus in their centre.

Look for developing blood vessels (*d*) between the fat cells. Note that some of the young connective tissue corpuscles seem to enlarge, branch, and anastomose, and then become hollowed out to form a system of capillary tubes (*see text*, p. 82). Before leaving the specimen, observe that these clusters of fat cells being formed in the cutis vera are very evidently merely a modification of the ordinary connective tissue of this part, with which they are continuous at their periphery.

(3.) *S Fat, hardened in alcohol, stained with hæmatoxylin. B.* (for envelope of fat cells).

Any specimen of fat which has been hardened in spirit will show the envelopes of the cells. Under the high power, observe the shape of the cells which, as they tend to collapse when the fat within them is removed, may be hexagonal or rectangular, apparently from mutual pressure. The envelopes are very distinctly seen as a darker border to the cells, the fat having been dissolved away. At some point within the envelope look for the nucleus, stained blue. When the outline of the cell is angular it is most frequently seen in one of the projections. In this specimen, even more clearly than in the first, the outlines of cells in a deeper plane can be very distinctly seen through those above them.

(4.) *Fat cells preserved in glycerine, teased in Farrant's solution.*

To see crystals of stearic, oleic, or palmitic acid, keep a small piece of fat for two or three days in glycerine; tease, and mount in Farrant.

Look for groups of acicular crystals in the centre of the cells. (*Fig. 24 A b.*)

(5.) *S Injected fat. B.*

Note the capillary network of the clusters of fat cells. Look for indications of the small afferent and efferent vessels. The specimen should be a thick rather than a thin one, and dehydration and clearing should be carried out in watch glasses.

(6.) *Section of lymph gland of ox, stained with hæmatoxylin or picro-carmin. F. or B. (Fig. 72.)*

Under the high power, examine the lymph sinus (*see "Lym-*

FIG. 25.

SECTION OF COSTAL CARTILAGE (HUMAN), STAINED WITH PICO-CARMINE
× 300.

- a.*—Hyaline matrix.
- b.*—Cartilage cells.
- c.*—Capsule of cartilage cell.
- d.*—Cell shrunken in preparation.
- e.*—Peri-capsular areola of lightly stained matrix.

FIG. 26.

SECTION OF INTERVERTEBRAL DISC OF SHEEP, STAINED WITH PICO-CARMINE × 300.

- a.*—White fibrous tissue.
- b.*—Group of cartilage cells.
- c.*—Cell showing branching process.

Fig. 25.

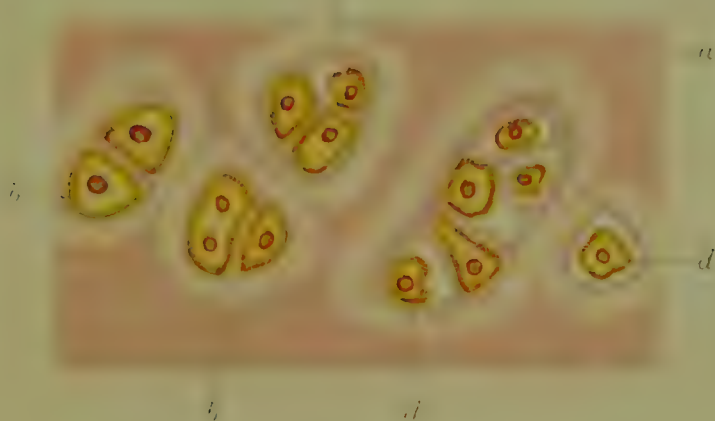
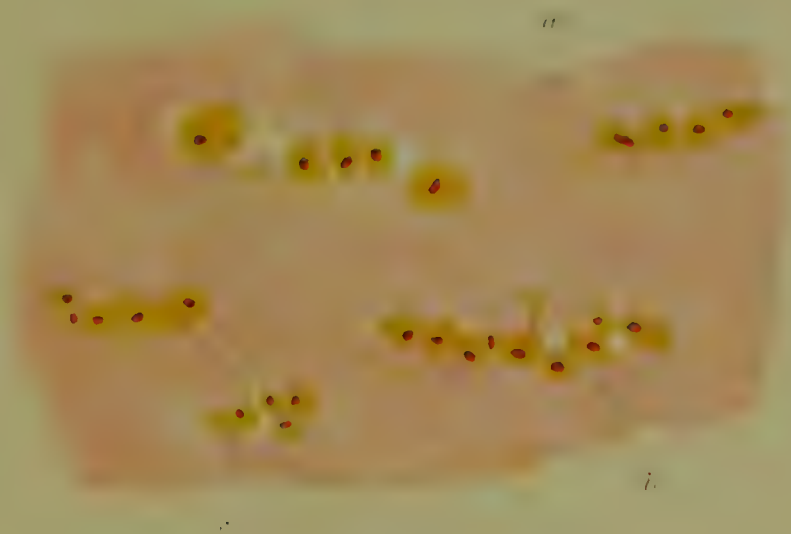


Fig. 26.



phatic gland”), and study the network of adenoid tissue (*d*) crossing it. Note the delicate, branching, nucleated cells anastomosing with each other, and forming a very beautiful reticulum, in the meshes of which lymph corpuscles can be seen. These are either numerous or few, according to the thickness of the section, and the way in which it has been handled after cutting. As a rule, it is well to see that the sections are thin, and that they experience a good deal of shaking in the water in which they are placed, as this helps to work out the superfluity of lymph cells from the sinus, and in this way the adenoid network becomes revealed. Now observe the “lymphoid” or “lymph follicular” tissue (*a*) of the gland, and note that the lymph cells are here so crowded together that the network of cells and fibrils is quite obscured. It should be borne in mind, however, that the network is in itself much more delicate and less easily seen than that crossing the lymph sinus.

(7.) *T.S. Umbilical cord of fœtus, stained with carmine or hæmatoxylin. F. (Fig. 18 B.)*

It is well to use a cord from a young fœtus, as at birth the mucous structure of the jelly is beginning to be obscured by the appearance of fibres in the intercellular matrix.

Under the low power, identify the arteries and vein of the cord, seen here in transverse section; find a suitable part of the surrounding tissue, where the cells can be well seen, and put on the high power. Note the very various shapes of the nucleated cells, and their long processes, which may anastomose with those of adjacent cells, or become lost in the mucin matrix. Here and there, between the cells, note the presence of delicate filaments in the intercellular matrix, indicative of an approaching transformation to ordinary connective tissue. These filaments, it will be noted, are to be seen between the cells, and not in any way in continuity with them (*see* “Development of connective tissue”).

Very beautiful specimens of mucous cells may be seen in the young connective tissue in T.S. (Tail of young Salamander).

(8.) *Skin of web of frog's foot. B. (Fig. 18 A.)*

Excise the web of a frog's foot, separate the upper from the under skin, and mount one of them in balsam after dehydration and clearing in watch glasses. Under the high power, look for the appearance shown in *Fig. 18 A*, if the animal was exposed to full daylight, for sometime before its death. Note the branching pigment cells, with the pigment retracted, however, to the centre

of the cell, leaving the branches clear. The nucleus is not seen, as it is surrounded by the pigment. If the frog has previously been kept in the dark, the pigment would be found to have extended into the branches of the cells.

CARTILAGE.

Like other forms of connective tissue, cartilage consists of cells embedded in a matrix. The matrix varies in character, and there are three forms of cartilage found in the human body.

1.—*Hyaline.*

2.—*White fibro-cartilage.*

3.—*Yellow fibro-cartilage.*

1.—**Hyaline Cartilage** or *gristle*, as it is commonly termed, is found covering the articular ends of bones, to which it affords a smooth and elastic surface ; and as the costal cartilages, between the ends of the ribs and the sternum. The nasal and laryngeal cartilages, with the exception of the epiglottis and the cornicula laryngis, are of the hyaline variety, as are also the tracheal rings and the bronchial plates. Most of the bones of the body are preformed in hyaline cartilage, *i.e.*, are represented by a cartilaginous simulacrum in the embryo.

This form of cartilage is termed hyaline from the characters of the matrix, or ground substance. In many cases it is clear, homogeneous, and translucent, though in articular cartilage it presents somewhat the appearance of ground glass. In old age patches of fibrillation frequently become visible, due to a deposit of calcareous salts.

The matrix is developed from the coalesced capsules of successive generations of cartilage cells, and in nature is probably closely allied to the gelatigenous element of ordinary connective tissue, the white fibres. It is said to be possible, by maceration, to disintegrate it into delicate fibrils, which, in the case of articular cartilage, run vertically to the surface, and in the costal cartilages in their long axis. Like the fibrils of white fibrous tissue, they are probably embedded in, and cemented together by, a mucinous ground substance, which, like that of ordinary connective tissue, reduces nitrate of silver. If a section of hyaline cartilage be treated with this reagent, the matrix is stained brown, and the spaces containing the cells, left clear. On staining with gold chloride, on the other hand, the cells are deeply affected, and the intercellular substance only slightly so.

The cells of hyaline cartilage are for the most part round, or oval, when they occur singly, but when they are grouped together, their neighbouring sides are flattened. In a similar manner, when they occur in rows rather than in groups, as in the deeper part of articular cartilage, the two ends of a cell which abut on those next in the chain are not rounded, but straight. Each cartilage cell is a nucleated protoplast, which entirely fills the space in the matrix in which it lies. This space is enclosed in a very well marked capsule, with a double contour, which is closely adherent to, or even fused with, the fibrillar ground substance. In some cases the fusion is so complete that no capsule can be differentiated as such ; but, as a rule, the distinction between it and the surrounding part can be clearly seen. The arrangement of the cartilage cells with reference to each other, varies with the locality from which the tissue was taken, and also in different parts of the same section. In a transverse section of *costal* cartilage, the whole may be seen to be surrounded by a layer of fibrous tissue, the perichondrium, composed of white fibrous laminae, with flattened connective tissue corpuscles between them, which appear fusiform in section. Immediately beneath the perichondrium, the cartilage cells are small, and flattened like the connective tissue cells, conformably with the surface, and occur singly rather than in groups. No sharp line of demarcation can be drawn between the perichondrium and the subjacent cartilage, the fibrous laminae of the former gradually fading away in the matrix of the latter, and a gradual transition taking place from connective tissue to cartilage cells. As the surface is receded from, the cells assume a more typical appearance, being less flattened (*i.e.*, fusiform in section), exhibiting a distinct capsule, and often occurring in pairs. Still further from the surface, the cells become larger, rounder, and are either arranged irregularly, or in groups. Towards the centre, the cells are usually in groups consisting of two or three, or, more frequently, a greater number.

This grouping results from the method of development and growth of the tissue. In an early stage the cells lie in close proximity to each other, separated by very little intercellular substance, the matrix being subsequently formed between them by the fusion of the capsules of successive generations of cartilage cells. Thus a cell will "secrete" a capsule, and will then divide into two daughter cells. Each of these, in turn secretes

FIG. 27.

SECTION OF CARTILAGINOUS NASAL SEPTUM OF KITTEN, STAINED
WITH Picro-Carmine $\times 300$.

- a.*—Perichondrium.
- b.*—Hyaline matrix.

FIG. 28.

V.S. ARTICULAR CARTILAGE OF DOG, STAINED WITH Picro-Carmine
 $\times 300$.

- A.—Hyaline cartilage.
- B.— „ „ calcified.
- C.—Bone.
- a.*—Flattened cells near surface of cartilage.
- b.*—More deeply placed rounded cells.
- c.*—Cells arranged in vertical rows.

Fig. 27.

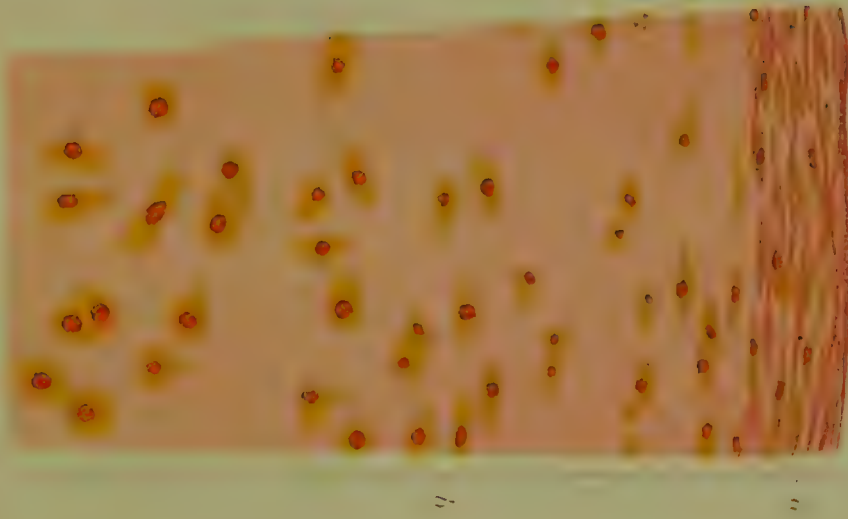
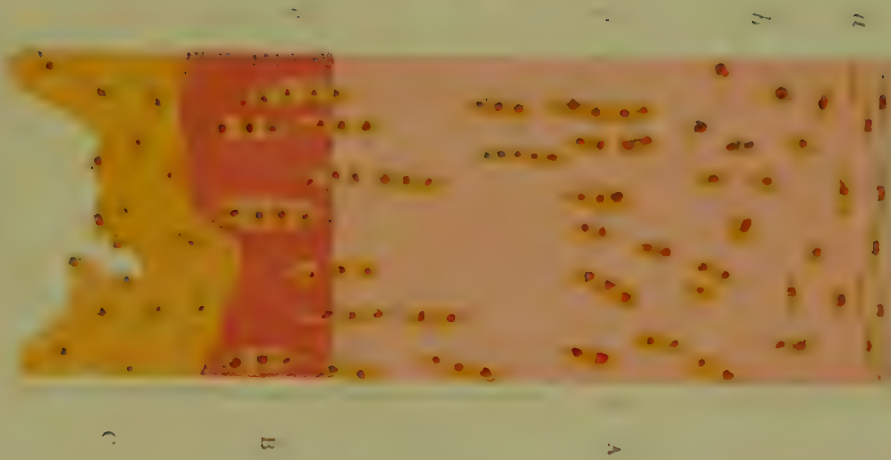


Fig. 28.



its own capsule around it, so that they become separated from each other by an intervening double sheet of (future) matrix, while the original capsule of the parent cell loses its identity, and fuses with the matrix already present. The two daughter cells now present an appearance frequently seen in ordinary costal cartilage, viz., two cells in close proximity, each enclosed in its own capsule, and the two capsules flattened along the line of contact with each other, *i.e.*, the line of division of the parent cell. But the process does not stop here. Each of the cells divides in turn into two, in a similar manner, so that we have now a group of four, each of which secretes a new capsule around it. In this case the lines of division are again straight, so that the four cells only present a rounded outline where they take part in the formation of the general periphery of the group. Though all but the most recently produced capsules rapidly fuse with the matrix, it is often possible to distinguish three generations of capsules in one group. (*Fig. 25.*)

In a vertical section of *articular* cartilage (*Fig. 28*), the cells are arranged in rather a different manner. Immediately beneath the surface, *i.e.*, next to the cavity of the joint, they are flattened as in the case of costal cartilage, and lie in the surface plane. A little deeper, they become rounded and more irregular in disposition, until the principal part of the cartilage is reached, where they are arranged in rows vertical to the surface. These chains of cells contain a varying number in each. Three, four, five, six or more may unite to form one row. The fact that articular cartilage readily splits, in a line vertical to its surface, is perhaps attributable to the disposition of these cells. In adult life the cartilage immediately in contact with the bone undergoes calcification, so that a more granular, deeply stained layer, differentiated sharply from the rest by a wavy line, is here to be looked for. In young articular cartilage this calcified area is not found. At the edges of the articular surface, the cartilage does not end abruptly, but a gradual transition takes place between it and the fibrous layer of periosteum of the bone.

Growth of Hyaline Cartilage.—In addition to the formation of the matrix through the coalescence of the successively produced capsules of the cells as already described, the cartilage grows by continuous transformation of the deeper part of the perichondrium or investing fibrous membrane, and it is especially to this, that the increase in size of the costal cartilages during adolescent

life is due. The transformation is not unlike that which takes place in the deeper layers of the periosteum in the case of bone. The fibrous lamellæ of the perichondrium become directly converted to hyaline matrix, and fuse with that already produced, while the cells assume the characters of cartilage cells.

A point of some interest in the development of cartilage is, that, in a comparatively early stage, the cells are irregular in shape rather than round, and are not arranged in groups, but scattered indefinitely through the matrix. A somewhat similar condition, as far as the shape of the cells is concerned, is permanent in the cartilage of some cephalopods, such as the cuttlefish and common squid; but here the cells are very definitely grouped. *Fig. 30* shows two of these groups, and it will be noted how the cells are flattened towards each other, and from their rounded peripheral surface, give off well defined processes to anastomose with the cells of neighbouring groups. The canaliculi in which the processes lie, afford a ready means for the percolation of lymph throughout the tissue for its nutrition, but they have not yet been shown to exist in human hyaline cartilage. It is possible that they may be there in a much more delicate form, and in support of such a theory it may be mentioned that communicating channels between the cells may certainly be traced in the white fibro-cartilage of the intervertebral disc. At the same time, it may be borne in mind that this tissue partakes at least as much of the nature of ordinary fibrous tissue or tendon as it does of cartilage, with which it must not be too closely identified, though it seems to be sufficiently related to it to be placed under the same general heading. The nutrition of hyaline cartilage must, in any case, take place very slowly. The perichondrium contains blood-vessels and lymphatics, but none enter the substance of the cartilage itself. In some manner, therefore, which is not yet determined, the lymph from the vessels of the perichondrium presumably percolates through the matrix of the cartilage, it may be by means of exceedingly delicate canaliculi passing between the cell spaces.

2.—White Fibro-Cartilage.—This is found in the intervertebral discs, forming the menisci of joints, such as the temporo-maxillary and the sterno-clavicular, deepening the cavity of the hip and shoulder joints, lining the grooves in bones for tendons, forming sesamoid bones, etc. It varies somewhat in character in

different places. In all, it belongs obviously to the class of fibrous connective tissues as much as to that of cartilage proper, but the proportion of the two kinds of tissue to each other varies greatly. In the *intervertebral disc*, the outer part is almost entirely fibrous, the cartilage cells only appearing as the centre of the disc is approached. The centre itself is neither cartilaginous nor of the nature of fibrous connective tissue, but consists of an exceedingly delicate reticulum, representing the remains of the *chorda dorsalis*. The cartilaginous part of the disc surrounding the central chordal portion is composed of true cartilage cells, surrounded by their capsules and a certain amount of hyaline matrix, embedded amongst bundles of fibrous tissue of the tendinous type. The fibrils are distinctly wavy in their course, as may be seen in *Fig. 26*. These fibrils are no doubt chemically and histologically similar to those of the fibres of ordinary connective tissue.

The cells are usually round or oval in shape, and occur either singly, or in groups of two or three. The individual cells are often surrounded by concentric rings, due to the production of successive capsules, thus acquiring somewhat the appearance presented by a starch granule. Calcareous particles are often to be observed outside the capsules of the cells.

Processes can frequently be traced from the cells into the surrounding matrix, especially in specimens stained with chloride of gold, but they are more obviously seen in those parts of the disc where the transition between connective tissue and cartilage is taking place, than in the part immediately around the central pulp. The processes are often of considerable size, giving off branches and anastomosing with those of neighbouring cells.

The fibro-cartilaginous disc is connected to the bone of the vertebra above and below it, by a thin layer of ordinary hyaline cartilage. A similar layer also intervenes between the disc and the bone on either side of it, in the sacro-iliac and pubic symphyses.

The *semilunar cartilages* of the knee-joint are somewhat different in character. There, the tissue is much more distinctly of the fibrous or tendinous type, and the cells are smaller than those of ordinary cartilages. They are scattered irregularly throughout the fibrillar matrix. They possess a hyaline capsule, which is not, however, so obvious as that of the cells of the cartilaginous part of the intervertebral discs.

3.—**Yellow fibro-cartilage** is found in the cornicula laryngis, the epiglottis, the external ear, and the Eustachian tube.

Its structure is very well seen in the arytenoid cartilage of the sheep, which is partly hyaline and partly elastic. Under the microscope, as the hyaline area is left and the elastic approached, isolated fibres of elastin are to be seen appearing in the hyaline matrix between the cells, but entirely unconnected with them. These fibres anastomose to form a network, and increase in number and density, till a felted framework is formed, which occupies the whole of the intercellular space, with the exception of a narrow ring of hyaline matrix, which is left around each cartilage cell. The elastin sometimes appears rather in a granular than in a reticular form, from the fact that the fibres have been cut transversely; or, in some cases, from its deposition in granules which have not yet run together to form fibres. In elastic cartilage we have, in reality, a basis of hyaline matrix in which a dense network of elastic fibres becomes developed, the original matrix persisting in the meshes of the network and around the cartilage cells. (*Fig. 29.*)

Examine the following specimens:—

(1.) *Cellular cartilage of ear of mouse, stained with hæmatoxylin. B.*

Remove the skin from the ear of a mouse, stain a thin piece of the cartilage in hæmatoxylin, and mount in balsam. Observe under the high power the cartilage cells, which are round, or polygonal from mutual pressure. There is no matrix, such as occurs in hyaline cartilage, between the cells, which are merely separated from each other by cement substance. On focussing, the outlines of cells, in different layers, come into view. Both the cell protoplasm and the nucleus seem to have disappeared, leaving the cells clear, not granular. Cellular cartilage does not occur in the human body.

(2.) *T.S. Costal cartilage (human), stained with picro-carmin. F. (Fig. 25.)*

Under the low power, observe the perichondrium or fibrous investment of the cartilage, stained deeply with the carmine. Note the general disposition and shape of the cartilage cells—small and flattened at the surface beneath the perichondrium, larger, more rounded, and in groups toward the centre. These groups usually have the appearance of being surrounded with a halo (*e*) of some breadth—the more recently produced matrix—

which does not stain so deeply as the rest with the reagent. Here and there, especially towards the centre of the section, look for patches of calcification, deeply stained with the carmine and showing very distinct fibrillation.

Under the high power, study first the perichondrium with its laminae of fibrous tissue, with connective tissue cells between them, which appear fusiform in section; note the transition between the fibrous tissue and the clear cartilage matrix beneath it, and between the cells of the two tissues. Now select any small group of cartilage cells which shows the capsules distinctly, and examine carefully. Note the granular cells themselves in most cases shrunken within the capsules, their remains collected around the nucleus (*d*). Enclosing the cells, look for the double contour of their individual capsules, and also for that of the parent cell from which they were derived (*c*). Two or three generations of capsules can usually be made out with a little care. Observe again, under this power, the halo of faintly stained ground-substance surrounding the groups, representing the series of capsules, now indistinguishable from each other, which have last fused with the general matrix. Examine a calcified patch, and note the deep staining and distinct fibrillation; between the fibrils rows of calcareous particles may sometimes be seen.

Stain a section similar to the above in $\frac{1}{2}$ or 1 per cent. solution of osmic acid for an hour, and mount in Farrant's solution. Small oil globules, stained black by the reagent, may be looked for under the high power, in the protoplasm of the cartilage cells. They indicate a degenerative process, and, like the fibrillation, is especially to be observed in the cartilage of old people.

(3.) *Hyaline cartilage of frog, stained with nitrate of silver.* B.

Rub the cartilage of the head of a frog's femur with solid silver nitrate; expose to light in spirit and water; cut sections by hand with a razor, and mount in balsam or Farrant.

Observe the matrix, stained brown with the silver nitrate, which has become reduced, and the cell spaces left clear. The staining is similar to that of ordinary connective tissue by the same reagent, and constitutes a "negative" image of which the following may be considered the "positive."

(4.) *Hyaline cartilage of frog, stained with gold chloride.* B.

Treat the head of a frog's femur with gold chloride by the

FIG. 29.

SECTION OF EPIGLOTTIS OF SHEEP (YELLOW ELASTIC CARTILAGE),
STAINED WITH PICRO-CARMINE $\times 300$.

- a.*—Perichondrium.
- b.*—Cartilage.
- c.*—Cartilage cell.
- d.*—Elastic tissue.

FIG. 30

SECTION OF CARTILAGE OF SQUID, STAINED WITH PICRO-CARMINE
 $\times 350$.

- a.*—Hyaline matrix.
- b.*—Branching cells.
- c.*—Cell slightly shrunken in hardening.

Fig. 29.



Fig. 30.



boiled formic acid method. Cut sections by hand, and mount in balsam or Farrant.

Note the "positive" image—the cells being deeply stained, while the matrix is comparatively neglected. The cells here do not tend to shrink within their capsules, as in the case of costal cartilage (2), of which the shrinkage of the cells is rather a peculiarity.

(5.) *V.S. Articular cartilage of cat or dog, stained with picrocarmine. F. (Fig. 28.)*

Under the low power, observe three principal layers of tissue :

(A) Hyaline cartilage, constituting the greater part of the cartilaginous covering.

(B) Calcified hyaline cartilage. This is the deeper part of the previous layer in reality, which has become the seat of deposition of calcareous salts. It is narrower, more granular, and more deeply stained than the layer of uncalcified cartilage above it. The two are distinctly demarcated from each other by a wavy line.

(C) Bone—the extremity of the epiphysis.

In (A) observe the hyaline matrix and the different disposition of the cells at different levels. Near the surface (*a*) they are small and flattened, and it will be noted that there is no perichondrium here, the cartilage ending abruptly, and affording a smooth surface for the opposing cartilage to move upon. Deeper, the cells lose their flattened form, and are more irregularly placed (*b*), while in the main body of the cartilage they are arranged in vertical rows (*c*), which also extend through the calcified area, up to the margin of the bone. Observe that the bone is of the cancellated kind which constitutes the epiphyses in general. Note that the cancelli of the bone communicate by channels with the lower rows of cartilage cells at the margin of the calcified cartilage. The purpose of this is rather obscure, but that they do so is readily verified in an ordinary specimen.

Under the high power, examine the above structures more minutely. Trace the cartilage round to the periphery, where it joins the periosteum of the bone, and study the transition between the two kinds of tissue. Examine the vertical rows of cartilage cells, which are so characteristic of articular cartilage ; the calcified area ; and the bone. The calcified area is only to be seen in mature animals ; in the young, the hyaline matrix in an unaltered condition extends to the bone.

(6,) *Costal cartilage of child, stained with hæmatoxylin.* F.

Note the smallness of the cartilage cells, their irregular shape, and that they are not arranged in groups but scattered indefinitely over the field.

(7,) *Section of cartilage of squid, stained with picro-carmin.* F. (Fig. 30.)

Under the high power, identify the appearances of the groups of cells; especially note that the cells of the same group do not appear to communicate with each other by processes, but do so freely with the cells of neighbouring groups. In these embryonic forms of hyaline cartilage, capsules to the cells, such as those seen in adult costal cartilage, are not to be made out.

(8,) *Section of intervertebral disc of sheep, stained with picro-carmin.* F. (Fig. 26.)

Separate the intervertebral disc of a sheep from the adjacent vertebræ. Cut it into four pieces by a crucial incision, harden, and make sections of one of these in the plane of the disc. Under the low power, note the difference in general appearance between the more central cartilaginous part of the disc bounded by the rectangle, and the fibrous peripheral portion. In the latter, observe the arrangement of the fibrous tissue in alternate lamellæ—a lamella with fibres running longitudinally, having on each side of it one with fibres running transversely, or at some intermediate angle. Under the high power, identify the cartilage cells with their hyaline capsules often surrounded with calcareous particles, and the wavy, fibrous tissue (*a*) amongst which the cells are placed. Find a cell showing long branching processes, and endeavour to trace these until they anastomose with those of others. In the outer part of the disc, examine again the alternate arrangement of lamellæ.

(9,) *V.S. Intervertebral disc of cat (with osseous plate of vertebræ above and below), stained with picro-carmin.* F.

Examine the specimen under the low power for the general arrangement of the parts. Note the reticular pulp in the centre of the disc, representing the chorda dorsalis; the cartilaginous area; and beyond that, the fibrous lamellæ. Note that the disc is separated from the bone above and below it, by a thin layer of intervening hyaline cartilage.

(10,) *Section of semilunar cartilage of knee-joint, human, stained with picro-carmin.* F.

Under the high power, note the small size of the cartilage cells, their indefinite arrangement, and the narrowness of their capsules. The general appearance of the tissue is more fibrous than cartilaginous.

(11,) *V.S. Epiglottis of sheep, stained with picro-carmin.* F. or B. (Fig. 29.)

Under the low power, observe the stratified, squamous epithelium, surrounding the whole section except at its base, and the connective tissue stained pink, subjacent to it. In the layer of epithelium on the posterior surface, note the presence here and there of taste buds. Beneath the epithelium, especially on the other side, note the presence of numerous mucous glands with well-marked demilunes, which are particularly well seen in the specimen. Forming a core to the section, observe the plate of elastic cartilage, stained bright yellow by the picric acid, and surrounded with a border of deep pink, the perichondrium. Put on the high power over a portion of the elastic cartilage, and identify its structure as already described—the cartilage cells (*c*), round or oval in shape, nucleated, each surrounded with a layer of hyaline matrix, and outside this a dense feltwork, or sponge-like reticulum of elastic fibres. This intercellular network is so close in many cases as to form virtually a solid elastic mass (*d*). Now pass to the point of junction of the cartilage with the perichondrium. Here the bars of elastic matrix between the cells break up into fibres and fibrils, which become lost in the layer of hyaline cartilage upon which the perichondrium rests. Amongst the scattered elastic fibres, observe a number of small cells—transitional forms between the true cartilage cells and the connective tissue cells of the fibrous perichondrium; observe the perichondrium, similar in structure to that of hyaline cartilage.

(12,) *V.S. Arytenoid cartilage of sheep, stained with magenta.* F.

Magenta is a useful stain here, as it specially affects elastic fibres.

A similar appearance of elastic fibres in a hyaline matrix, to that noted beneath the perichondrium in the last specimen, is also to be seen here. The lower, broad part of the section, shows

hyaline cartilage alone, the upper conical portion is elastic. Under the microscope, on passing from the hyaline to the elastic area, note the gradual appearance of elastic fibres, stained red with the magenta, in the matrix, particularly in the neighbourhood of the cartilage cells; and that, as the purely elastic part is reached, these fibres become increased in number to such an extent that they form a dense network, occupying the whole of the intercellular area, with the exception of a narrow layer of persisting hyaline matrix around the cells themselves.

APPENDIX TO CHAPTER V.

METHODS OF PREPARATION.

1. **Human skin. Osmic Acid and Picro-carmin.**—Harden in Müller's fluid, and transfer, after washing, to 1 per cent. solution of osmic acid for twenty-four to forty-eight hours. Cut in gum; stain in picro-carmin; and mount in Farrant. Or, instead of staining in bulk, sections may be placed in osmic acid for a few minutes after cutting.
2. **Fœtal scalp for developing fat cells.**—Same as 1.
3. **Injected fat.**—Remove a small piece of fat from an animal which has been successfully injected from the aorta with carmin or blue gelatine mass. Harden in Müller's fluid; cut in gum, or preferably in paraffin—the sections should be thick; dehydrate, and clear, in watch-glasses. Mount in balsam.
4. **Lymph gland for adenoid tissue.**—Harden the lymph gland of an ox or a sheep in Müller's fluid and spirit; stain in picro-carmin or hæmatoxylin, and mount in Farrant or balsam. Or, stain in bulk in borax carmin; cut in paraffin; mount in balsam.
5. **Umbilical cord for mucous tissue.**—Harden the cord of a 3-5 months fœtus in Müller's fluid and spirit; cut in gum; stain in picro-carmin or hæmatoxylin; mount in Farrant.
6. **Costal cartilage (human, adult).**—Harden in Müller's fluid; cut in gum; stain in picro-carmin, hæmatoxylin, picric acid, osmic acid, carmin or magenta; mount in Farrant.
7. **Costal cartilage of child.**—Harden in Müller's fluid; cut in gum; stain in picro-carmin or hæmatoxylin; mount in Farrant.
8. **Articular cartilage.**—Select the head of one of the long bones of a cat or dog which is distinctly old rather than young, in order to have a well marked, calcified area. Soften in chromic and nitric fluid; harden for a few days in spirit; cut in gum; stain in picro-carmin, osmic acid, or hæmatoxylin; mount in Farrant.
9. **Cartilage of squid.**—Harden in Müller's fluid; cut in gum; stain in picro-carmin; mount in Farrant.
10. **Intervertebral disc of sheep.**—Get a portion of the spinal column of a sheep, including three or four vertebræ. Saw the vertebræ transversely through their middle, and place the resulting pieces in chromic and nitric fluid till the bone is softened. Now excise the discs and divide them into four parts each, by a crucial incision. Harden in Müller's fluid: cut in gum; stain in picro-carmin or hæmatoxylin, and mount in Farrant or

balsam. It is better not to use spirit in hardening, as the tissue is very liable to become over-hardened.

11. **Intervertebral disc of cat.**—Place a piece of the spinal column of a cat in chromic and nitric fluid. When the bone is softened divide transversely across the middle of the bodies of the vertebræ. Harden in Müller's fluid; cut in gum (in long axis of column); stain in picro-carmin or hæmatoxylin; mount in Farrant or balsam.

12. **Semilunar cartilage of knee (human).**—Harden in Müller's fluid; cut in gum; stain in picro-carmin; mount in Farrant or balsam. Spirit should not be used in hardening, for the same reason as in case 10.

13. **Epiglottis of sheep.**—Harden in Müller and spirit; cut in gum; stain in picro-carmin; mount in Farrant or balsam.

14. **Arytenoid cartilage of sheep.**—Harden in Müller, or Müller and spirit; cut in gum; stain in picro-carmin, and mount in Farrant; or stain in magenta, and mount in Farrant or balsam.

CHAPTER VI.

*THE SIMPLE TISSUES (Continued).***BONE.**

THERE are two varieties of bone, the compact and the cancellous. Compact bone is heavy, and virtually solid, except for the presence in it of very fine canals, the Haversian canals, which are apparent to the naked eye, but can be more easily distinguished by means of a lens. Cancellous or "spongy" bone, on the other hand, is extremely light, and so far from being solid, is composed of more or less delicate trabeculæ or plates of osseous tissue bounding comparatively large spaces or cancelli.

Compact bone is found forming the outer and greater part of the shaft of long bones, in fact, all but the layer of bone immediately surrounding the medullary cavity; the hard outer shell of the short or cuboidal bones; the greater part of the irregular bones; the inner and outer tables of the skull.

Cancellous bone forms the innermost portion of the shafts of long bones, *i.e.*, the layer immediately bounding the medullary cavity; the ends of long bones; all but the shell of short bones; the diploë of the flat bones of the skull; the inner part of the irregular bones.

Bone consists essentially, whether compact or cancellous, of the two ordinary elements of connective tissue, namely, cells and a matrix. The cells are called *bone corpuscles*, and present a characteristic appearance; the matrix is fibrous and arranged in *lamellæ* which have become calcified.

A bone may be thus said to consist of a structural or organic portion (the cells and the fibrous portion of the matrix), and a structureless or inorganic portion (the calcareous salts deposited in the matrix).

If it be placed in weak hydrochloric or nitric acid or nitro-

chromic fluid, it loses the salts deposited in the laminæ of fibrous tissue, to which it owes its hardness. After their removal in this manner the bone entirely loses its rigidity. It may be bent in any direction, and in the case of a long bone, such as the ulna or radius, can easily be tied in a knot, and on being untied, will resume to a great extent its original form.

It can also be cut readily with a knife, and it is from pieces of bone which have been thus decalcified that sections are usually made.

If, however, a bone be placed in the fire until it is completely charred, all the organic structural elements are, of course, burnt off, leaving only the inorganic structureless portion, consisting of calcium and other salts, in which case we have a chalky brittle representation of the original organ.

Again, the bone may be macerated in the ordinary manner employed in preparing it for anatomical purposes, and in this case we have remaining the calcareous and also the fibrous matrix; though this latter is in a dried state. All the other soft parts have been removed, namely, the periosteum, the contents of the medullary canal, medullary spaces, Haversian canals and lacunæ. Sections of such a bone are not cut in the microtome, but a thick section cut by other means is ground down to the requisite thinness.

The study of the structure of bone may be most conveniently commenced by the consideration of an unsoftened section, *i.e.*, one prepared in the manner last described, from which the soft parts have been removed, but which has not been exposed to the action of an acid. In this way we are able to study the form of the lacunæ and canaliculi in which the bone corpuscles were situated, and the relative arrangement of them, and the lamellæ and the Haversian canals. When these have all been observed, the soft parts can be studied in a section of softened bone.

The compact portion of the shaft of a long bone is traversed throughout its length by a system of tubes running longitudinally; anastomosing freely with each other; communicating on the one hand with the medullary cavity, and on the other with the surface of the bone beneath the periosteum or fibrous covering. (*Fig. 34.*) The tubes, or *Haversian canals*, in the recent state contain the blood-vessels, lymphatics and nerves supplying the tissue. But in a transverse section of an unsoftened long bone (*Fig. 31.*), these canals are everywhere seen as round spaces, either empty,

or filled with dust, etc., which has entered during the process of wearing down to the requisite thinness ; in which latter case they appear black. Surrounding each of these, may be seen a series of concentric lines extending to a variable distance. These are sections of the lamellæ of calcified fibrous tissue, and amongst them may be seen the lacunæ, or cell spaces, which contained in the recent state the bone corpuscles. The nature of these lacunæ, with the canaliculi passing from them, is very obvious in a dried specimen, as they are both filled with air, causing them to appear black, and consequently to stand out sharply from the colourless matrix in which they are placed.

In addition to the Haversian lamellæ, there may be seen in such a section, lamellæ surrounding the central cavity of the bone, and hence called medullary ; others at the periphery, peripheric, or circumferential ; and others between the Haversian systems, the intermediate lamellæ.

Systems of Lamellæ.—Thus, in a transverse section of a long bone, four systems of lamellæ can be seen :—

- (1,) *Peripheric.*
- (2,) *Haversian.*
- (3,) *Intermediate.*
- (4,) *Medullary or peri-medullary.*

In all these situations the nature of the lamellæ is the same, and the name given to them is merely indicative of their position.

(1,) *The Peripheric lamellæ.* As the name implies, these are the lamellæ found in several layers at the surface of the bone, immediately beneath the periosteum. Each lamella surrounds at least a considerable portion of the shaft.

(2,) *The Haversian lamellæ* are those surrounding the Haversian canals, forming, with them and their contents, the Haversian systems. In an ordinary unsoftened section of bone it is often difficult to discern precisely the outer margin of one of these ; but it is possible, by treating a specimen with nitrate of silver, to define their limits with great accuracy (*Fig. 31*).

(3,) *The Intermediate.* Everywhere between the Haversian systems, often abutting against their outer edge and terminating abruptly, there are to be seen other systems of lamellæ running in various directions. They are the remains of Haversian systems previously formed, which have been absorbed to make room for younger ones ; and also of earlier peripheric lamellæ. Often, instead of merely a few parallel lamellæ being seen, the

FIG. 31.

T.S. LONG BONE OF GIRAFFE, STAINED WITH NITRATE OF SILVER
× 100.

- a.*—Haversian systems the exact limits of which are defined by the reagent.
- b.*—Intermediate lamellæ, *i.e.*, remains of old Haversian systems and peripheric lamellæ.
- c.*—Haversian systems which have been recently laid down and consequently stain more deeply.

FIG. 32.

BONE LACUNÆ AND CANALICULI FROM SECTION OF CARAPACE OF
TORTOISE, UNSTAINED × 350.

- a.*—Lacuna.
- b.*—Canaliculi.

Fig. 31.

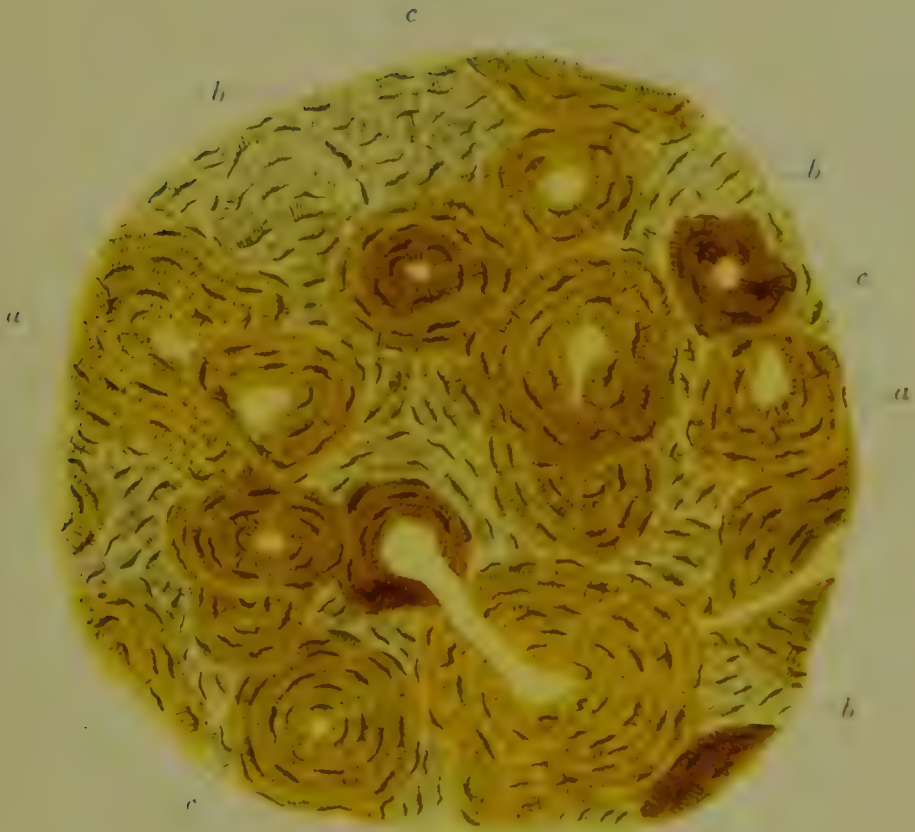
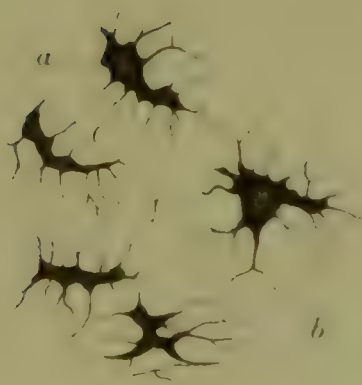


Fig. 32.



greater part of one of these original Haversian systems may remain.

(4.) *Peri-medullary*. As the name implies, these are the lamellæ surrounding the medullary cavity in the same way as the peripheric lamellæ surround the bone on the outside.

Structure of the Lamellæ.—The lamellæ are probably of the nature of white fibrous tissue which has undergone calcification. The fibrils constituting a lamella run parallel with each other, and usually straight; those of adjacent lamellæ often cross each other at an angle, so that when one is cut in a direction transverse to the axis of its fibres, the lamellæ on either side of it are so arranged that the fibres forming them are cut more or less longitudinally. Through this relative disposition of the lamellæ we are the better able to distinguish them from one another.

In addition to the fibres forming the lamellæ themselves, there are others—*perforating*, or *Sharpey's fibres*—to be found in bone. They pierce the lamellæ at right angles to their surface, and thus serve the purpose of binding them together in much the same way in which a nail would bind together several superimposed thin boards of wood. These perforating fibres, which have undergone calcification, are usually of the nature of white fibrous tissue; more rarely, they are elastic. They are very numerous in the peripheric lamellæ, but do not occur in the Haversian systems, the lamellæ of which they do not pierce; the reason of this will be apparent when the development of bone is considered.

The Lacunæ and Canaliculi.—Although in a section of unsoftened bone there are no bone cells present, the branched cell-spaces or lacunæ in which they lie, and which correspond to them in shape, stand out in marked relief, due to the fact that they are filled with air.

It is very easy to satisfy one's self that it is air, and not extraneous material acquired in grinding the specimen down to the requisite thinness, which occupies the lacunæ and canaliculi. If a section of softened bone be dehydrated with absolute alcohol, and allowed to become perfectly dry; then mounted in Canada balsam and examined; the lacunæ and canaliculi present the same appearance as in the unsoftened specimen. This is due to the fact that the Canada balsam does not immediately displace the air which entered the cell spaces when the specimen was dried. In a few hours, however, the air becomes absorbed, the

balsam takes its place, and the appearance described is lost ; but, while it lasts, it is precisely similar to that seen in unsoftened bone.

The lacunæ, or cell-spaces, lie between the lamellæ, and in section appear dark fusiform bodies with fine processes (canals) passing from them through the lamellæ to anastomose with the canaliculi of lacunæ in other planes (*Figs. 31 and 32*). They are not, however, fusiform in reality ; they appear equally so in longitudinal section, and are really cell-spaces flattened in shape in accordance with their position between the lamellæ. The canaliculi form a system of fine canals completely traversing the bone, and act as channels for conveying nourishment to every part of it.

The canaliculi of those lacunæ in the plane nearest to an Haversian canal, open into it. Those in the plane nearest the medullary cavity open into the medullary cavity. The canaliculi of the lacunæ at the periphery of an Haversian system anastomose sometimes with those of neighbouring systems ; more frequently, however, they turn back into their own, and have hence been called "recurrent."

Bone Corpuscles.—Although the bone corpuscles have disappeared from a dried, unsoftened section of bone, they may be described at the same time as the lacunæ, which they occupy. They are flattened, nucleated cells, with many delicate branches, which are prolonged into the canaliculi. The lacunæ and canaliculi are said to be lined with a delicate membrane, which may be separated along with the enclosed corpuscle.

The Periosteum, or fibrous investment of the bone, consists of two layers of connective tissue, an outer fibrous or protective layer, and an inner, which in the adult, is merely a looser, more delicate one, but in young, growing bone, the osteo-genetic or bone-producing layer (*see* "Developing bone"). Both layers of the periosteum contain blood-vessels, nerves and lymphatics, intended for the nutrition of the bone, which they enter through the Haversian canals opening on the surface, accompanied with a small amount of delicate connective tissue. In the young bone these are accompanied by numerous osteoblasts from the osteogenetic or deep layer of periosteum.

The Marrow.—This is the name given to the contents of the medullary cavity, the cancelli, and some of the larger Haversian canals of bone. It is of two kinds—red and yellow. The latter

differs from the former in containing a large admixture of fat cells, which preponderate over the other elements of the tissue and give it its yellow colour. It consists chiefly of fat cells, blood-vessels, and delicate connective tissue. It is found in the shafts of long bones and in some of the larger cancelli. Red marrow is found in the cancelli generally, especially at the ends of long bones; in the short bones of the hand and foot; in the diploë of the cranial flat bones; in the clavicle, rib and sternum. It consists of delicate connective tissue, blood-vessels, a few fat cells, and marrow cells in large quantity. The vascular arrangement is remarkable for the presence of a system of intercommunicating wide venous sinuses with thin walls, into which the smaller terminal divisions of the 'nutrient' artery—which enters obliquely the centre of the shaft—directly open. The cells in the tissue outside the blood-vessels are of several varieties (*Fig. 35*).

(1.) *Marrow cells proper*.—These are the most numerous, and are very similar to leucocytes, but larger. Their protoplasm is finely granular, and they possess a single nucleus, which becomes more visible on the addition of acetic acid. They are capable of amœboid movement and multiply by indirect fission. Cells not otherwise dissimilar to these, are also to be seen with coloured granules in their perinuclear protoplasm.

(2.) *Erythroblasts*.—Smaller, coloured, nucleated cells, which are regarded as the descendants of the coloured corpuscles of the embryo.

(3.) *Myeloplaxes*.—Large multinucleated irregular masses of protoplasm, probably concerned with the removal of bone. They are comparatively few in number. There are also to be seen somewhat similar, smaller cells with the nucleus showing commencing division, or budding. *Fig. 35* shows three of these smaller cells. In one, the nucleus is fairly round; in the second, it shows commencing constriction at more than one point; in the third, it is partially subdivided into three portions or daughter nuclei which are, however, still united to each other. These cells with budding nuclei may be myeloplaxes in process of development.

Development of Bone.—Most of the bones of the body are represented, prior to the commencement of bone formation, by cartilage. Some, however, such as the flat bones of the skull, by membrane, for the most part of the nature of white fibrous

FIG. 33.

V.S. FRONTAL BONE OF MAN, UNSOFTENED $\times 8$.

- a.*—Tables of skull, *i.e.*, compact bone.
- b.*—Diploë, *i.e.*, cancellous bone.
- c.*—Haversian canals and systems.
- d.*—Haversian spaces.
- e.*—Cancelli.

FIG. 34.

L.S. SHAFT OF FEMUR OF MAN, UNSOFTENED $\times 300$.

- a.*—Haversian canal.
- b.*—Bone corpuscles.

Fig. 33.

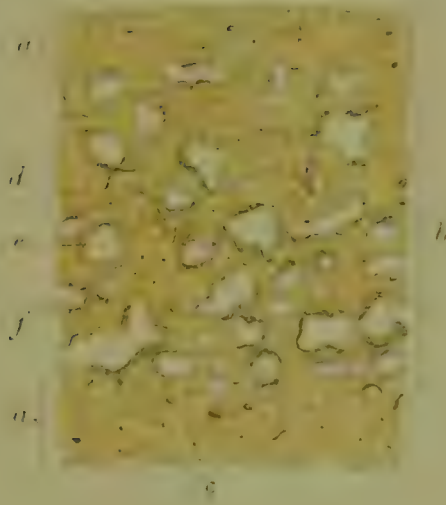
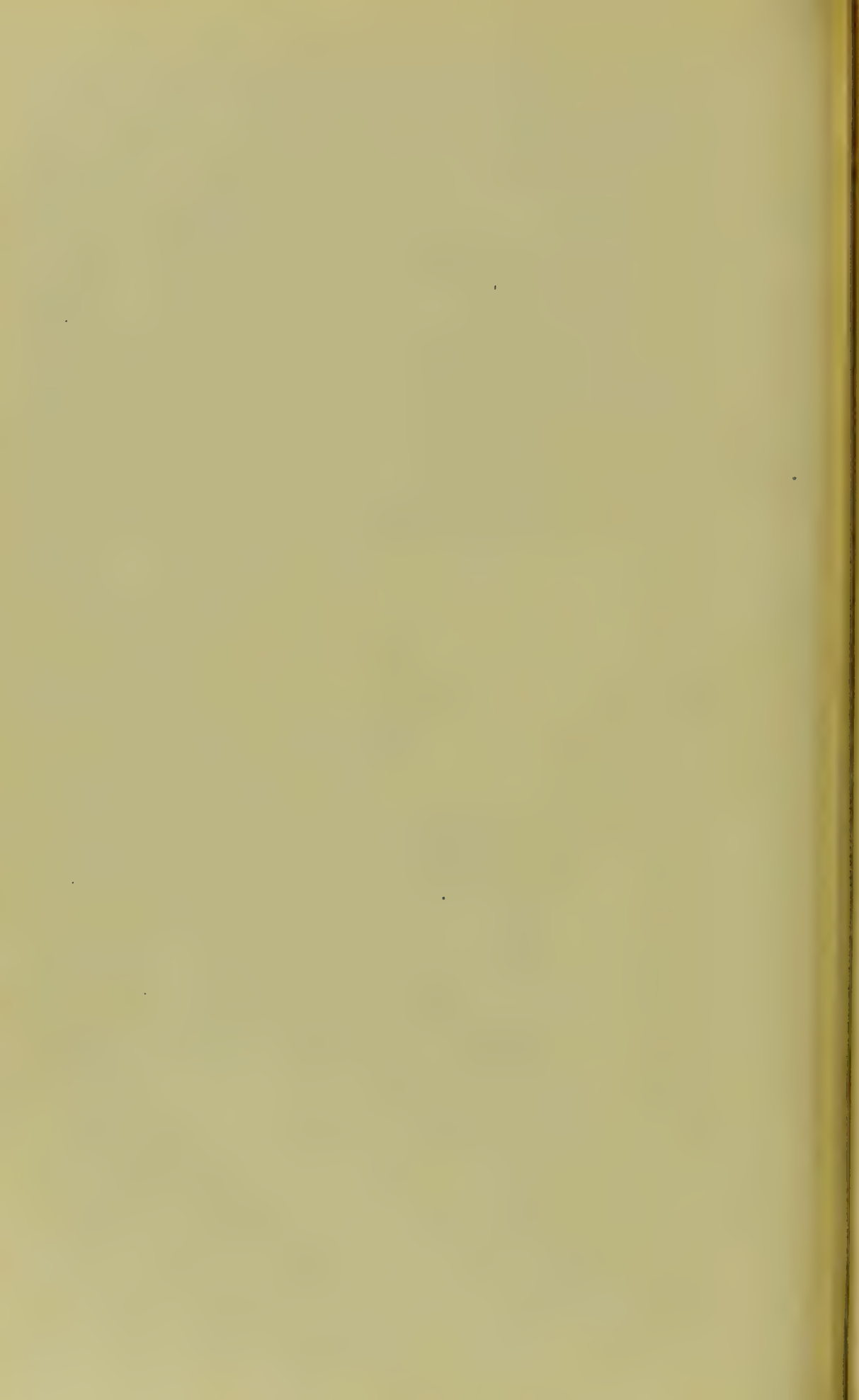


Fig. 34.





tissue. Thus, bone is said to be developed either in cartilage or membrane, but, in reality, the permanent tissue is only developed in the latter.

INTRA-CARTILAGINOUS BONE FORMATION.

All the bones of the body, with the exception of those of the vault and sides of the skull, and the facial bones, are represented in the embryo by a small cartilaginous simulacrum; and it is in and around this cartilage (hyaline) that the formation of bone commences. It will be convenient to give a short description of the process as it takes place in a long bone, such as the tibia or femur, and then to examine sections illustrating phases of the development. The two most necessary sections are a vertical one of the head of a developing long bone, such as one of the leg bones, and a transverse section of the same bone through the shaft. The age of the embryo should be such that a considerable amount of bone formation has already taken place, which is indicated by the unyielding character of the bone, and the difficulty in cutting it without previous softening by acid. To see every stage from the commencement, a series of sections of bones in different stages of development would, of course, be necessary.

The changes which occur in the cartilaginous simulacrum may be divided into the following stages:—

- (1,) *Proliferation*
- (2,) *Calcification*
- (3,) *Vascularisation*
- (4,) *Bone formation*

(1,) *Proliferation*.—When ossification is about to commence, the cells in the centre of the shaft increase in size, and proliferate; at first slowly, remaining separated by the newly-formed cartilage capsules; and then so rapidly that time is not allowed for the formation of individual capsules, and the cells come to lie in groups of a considerable number, in spaces which have been termed, “primary areolæ.” At the same time, the cartilage cells immediately above and below this area also commence to multiply by fission, dividing in such a way as to arrange themselves in vertical rows, parallel with the axis of the bone.

(2,) *Calcification*.—The next stage now commences ; calcareous salts being deposited in the cartilaginous trabeculæ bounding the primary areolar spaces, and also in the trabeculæ separating the vertical rows of cartilage cells. This deposition of calcareous salts renders the part affected much more easily stained, notably, for instance, by hæmatoxylin, the calcified cartilage usually standing out very distinctly when this reagent has been used. (*Figs. 38 and 41.*)

Coincident with these changes, an alteration has taken place in the perichondrium. This membrane has now assumed the character of periosteum, and from its deeper or osteo-genetic part, a layer of bone has commenced to be deposited on the outside of the cartilaginous rod. This sub-periosteal formation will be considered later, in detail ; at present it is sufficient to note the period of development at which it begins.

(3,) *Vascularisation*.—This commences with an eruption from the deeper osteo-genetic layer of the periosteum, towards the centre of the bone. Capillary blood-vessels, accompanied by delicate connective tissue and osteoblasts, penetrate the shaft midway between the ends, and advance towards the primary areolar spaces ; these, by the absorption of the intervening cartilage, they open into, and cause to communicate with each other, by their further progress. The cartilage cells filling these spaces either disappear, or, perhaps, take on a new function as osteoblasts. The larger spaces formed by the opening up of the primary areolæ are termed “secondary areolæ.” The capillary loops, connective tissue and osteoblasts, now proceed in a definite direction. They advance towards either end of the future bone, choosing for their path the longitudinal rows of cells, into the cavities of which they penetrate by absorption of the cartilage matrix between them ; that is to say, by absorption of the cartilage between the individual cells in the same row ; the cartilage matrix between the different rows of cells is not at present absorbed, but remains as calcified cartilage trabeculæ, on which temporary bone is to be laid down. (*Fig. 39.*)

(4,) *Bone formation* now commences, in connection with the remains of the calcified cartilage ; the osteoblasts accompanying the blood-vessels begin to deposit bone on the trabeculæ bounding the secondary areolar spaces in the centre of the shaft, and as the blood-vessels advance towards the ends of the shaft, the osteoblasts accompanying them also lay down bone on the surface of the cartilaginous trabeculæ, which separated the

vertical rows of cells. Inasmuch, however, as the process commenced at the middle of the shaft, the bone is always in thicker layers there, and grows thinner as it is traced upwards or downwards along the trabeculæ towards the limit which the blood-vessels have reached. (*Fig. 36 a.*)

Osteoblasts and Formation of Bone.—An osteoblast is a modified connective tissue corpuscle, that is to say, it is a connective tissue cell which, since its entrance with the blood-vessels into the cartilaginous fore-runner of the bone, has assumed a new function, that of secreting osseous tissue. At the point where bone is going to be deposited, the osteoblast attaches itself to the calcified cartilage, and by some process of solution, dissolves a portion of it, producing thus a small bay or hollow, in which it lies. It now secretes bone around it, and as it becomes submerged, acquires processes, and remains as a bone corpuscle. (*Fig. 42 g.*)

The bone thus laid down, has only a temporary function, and is ultimately all removed to make way for the medullary cavity. The two processes of construction and removal may be seen going on side by side. We have seen the part taken by osteoblasts in building up the temporary framework; the work of removal is carried out by osteoclasts. These are large, multinucleated masses of protoplasm, the origin of which is uncertain. They have, however, the power of dissolving bone and cartilage, and may be seen attached to the ends or sides of the bone-covered cartilaginous trabeculæ, which they are removing (*Figs. 39f and 42e*). As will be seen later, they also occur on the surface of bones which require 'modelling,' removing it in places, while the osteoblasts are adding to it in others.

Increase of the Bone in length.—As the process described above proceeds, from the centre to the ends of the bone, the cartilage cells in the layer immediately beyond what we may consider the *line of progression* of the blood-vessels, continue to divide and form vertical rows as before; that is to say, the cartilage continues to grow in the direction of the vertical axis of the bone, to compensate for the consumption of cartilage in the process of ossification. It is by the presence and activity of this, so to speak, 'cambium' layer of cartilage that the bone increases in length, just as the cambium ring enables a tree to increase in thickness.

The ossification of the epiphysis of a long bone commences

later than that of the shaft. After the epiphysial ossification has commenced, we have, therefore, the shaft in a more or less advanced stage of ossification; the epiphysis, with its central cells, commencing to proliferate and showing signs of commencing calcification; and between the two, the layer of growing cartilage, the 'cambium' zone. This zone persists until the growth of the bone is fully completed, when it disappears, and the epiphysis and shaft become firmly united. Thus it is possible to arrive at some idea as to the age of a bone at death, by observing the separability of the epiphyses from the diaphysis; in the case of the bones of young animals, the epiphyses readily separate from the shaft, before or after maceration, as they are only united to it by cartilage; in older animals they are more or less firmly united with bone, according to their age.

Periosteal Bone Formation.—We have now to return to what is, after all, the most important factor in the formation of a long bone, namely the periosteum; for it is from it that permanent bone is formed. The whole of the bone laid down in cartilage is absorbed, as the bone increases in width, in order to form the medullary cavity; and not only this, but the periosteal bone is also removed from the inside for the same purpose, as the bone grows; this removal being compensated by the continued growth of bone on the outside. The formation of bone by the periosteum is in reality the same process as ossification in membrane. As already stated, the periosteum consists of two layers, an outer fibrous or protective one, and an inner, cellular or osteogenetic layer. The outer is composed of lamellæ of fibrous tissue with flattened connective tissue cells placed between; the inner, of much looser, more delicate tissue, which is very cellular. Both layers contain blood-vessels, lymphatics, and nerves.

The first step in ossification is a direct calcification of the fibres (mainly white) in the deepest part of the osteogenetic layer next to the cartilaginous bone, accompanied by the assumption on the part of the cells between them of the function of osteoblasts (*Fig. 40*). These cells secrete around them, and upon the fibres between which they lie, osseous tissue, so that the innermost or deepest stratum becomes converted to bone, the osteoblasts becoming bone corpuscles. The process is continued in precisely the same way, the fibres becoming calcified from within outwards, and the cells proceeding to secrete bone in the same order;

the osteogenetic layer growing at the outside to compensate for the consumption within.

Formation of Haversian canals.—If the surface of a growing bone in transverse section be examined (*Fig. 40*), it will be seen that it has by no means an even outline. The formation of it is interrupted here and there, and an appearance is frequently presented of two ‘flying buttresses’ of bony tissue projecting into the periosteum and towards each other—evidently about to meet and enclose the space between them. The spaces thus enclosed are occupied by the tissue of the osteogenetic layer;—blood-vessels, nerves, and lymphatics, connective tissue, and osteoblasts; and will subsequently become Haversian canals by the deposition of concentric lamellæ from within. Before, however, this takes place, the space is enlarged by absorption, so that the size of the Haversian system resulting is larger than it otherwise would be. This enlargement of spaces previous to the laying down of fresh tissue results in the production of the intermediate lamellæ, as it takes place at the expense of the peripheric lamellæ, and any neighbouring Haversian systems, the remains of which constitute the intermediate lamellæ. This process of absorption from within, followed by subsequent replacement, continues throughout life in the case of the Haversian systems, the osseous tissue in this way being constantly renewed.

On the surface of the bone, when modelling is in process, osteoclasts, large multi-nucleated cells, similar to those which remove the bone from the inside of the shaft, may be observed. They may be seen here and there in the line of osteoblasts, lying in little bays or concavities on the surface of the bone, which are termed *Howship's foveoli*. These osteoclasts may be found in great numbers on the surface of irregular bones during the period of their growth.

INTRA-MEMBRANOUS BONE FORMATION.

The flat bones of the vault of the skull afford an instance of bone formation in membrane alone. The lower jaw, although originally represented by a bar of cartilage (Meckel's cartilage), is also formed in membrane around the cartilage, the latter taking no part in the process. In considering intra-membranous ossification, it has always to be borne in mind that all bone of a permanent character has this origin—

FIG. 35.

COVER-GLASS PREPARATION OF MARROW FROM BONE OF RABBIT,
STAINED WITH METHYL-BLUE $\times 350$.

- a.* —Small marrow cell.
- a*¹. — " " " budding.
- b.* —Large marrow cell.
- b*¹. — " " " with dividing nucleus.
- c.* —Cell containing reddish granules.
- d.* —Myeloplax.

FIG. 36A.

DIAGRAM OF DEVELOPING LONG BONE IN LONGITUDINAL SECTION,
TO SHOW GENERAL RELATION OF PARTS, STAINED WITH HÆMATOXYLIN
AND CARMINE $\times 20$.

- a.* —Calcified cartilage matrix.
- b.* —Periosteum.
- c.* —Periosteal bone.
- d.* —Endochondral bone.
- e.* —Rows of cartilage cells.
- f.* —Articular surface.

FIG. 36B.

L.S. FŒTAL PHALANX (HUMAN), STAINED WITH HÆMATOXYLIN $\times 10$.

- a.* —Periosteal bone.
- b.* —Remains of endochondral bone.
- c.* —Vertical rows of cells in cartilaginous head of bone.

Fig. 35.

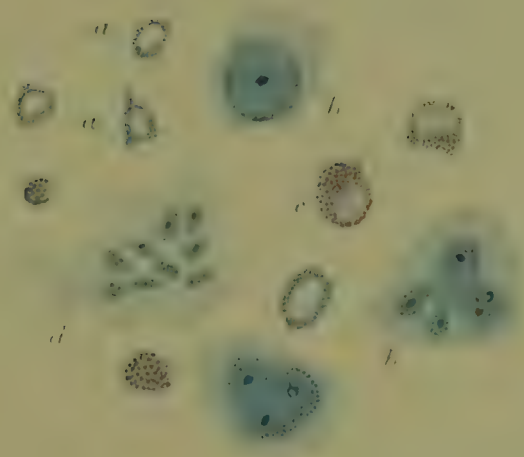
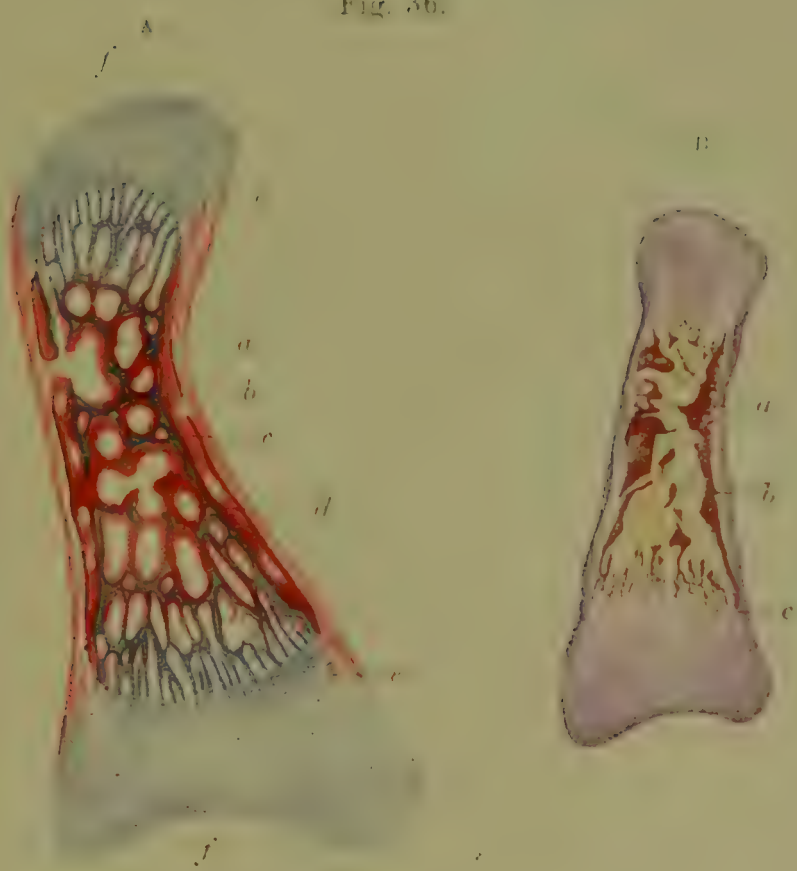


Fig. 36.



that periosteal bone formation is really intra-membranous. The ossification of one of the tabular bones of the skull, *e.g.*, the parietal, commences somewhere in the region of its centre. The structure of the membrane at this period is simply that of white fibrous tissue; that is, it is composed of white delicate fibrillated fibres, running in various directions in a ground substance, and between them are to be found many young connective tissue cells, the whole being traversed by a network of blood-vessels. The commencement of ossification is heralded by the direct calcification of the fibres at the centre by the deposition of calcareous salts in the interstitial substance uniting the fibrils; and the simultaneous assumption by the connective tissue cells of the function of osteoblasts. This, it will be noted, is precisely what occurs in the deep layer of the periosteum, when bone commences to be formed there; as in that case, the connective tissue corpuscles, now osteoblasts, secrete bone around themselves and upon the calcified fibres, remaining *in situ* as bone corpuscles. Ossification proceeds by the extension of this process outwards; and as the calcification and bone formation proceeds from the centre, the connective tissue grows at the periphery of the membrane. As in the case of the periosteal formation, the Haversian canals and systems result from the inclusion between the fibres, of blood-vessels, osteoblasts, etc. When the process has extended until nearly the whole membrane is ossified, the newly formed bone does not become continuous with adjacent ones; but as long as it, and they, are required to grow, there always remains a layer of growing connective tissue at the margin, the 'cambium' layer, feeding the bones which it separates, with connective tissue to supply their growth. The bones increase in thickness by the addition of successive layers beneath the periosteum, as in the case of the long bones already described; and while this is going on, alternate absorption and deposition are proceeding within, resulting in the opening up of the central part or diploe between the tables, to form cancellated bone.

Examine the following specimens:—

(1.) *T. S. Shaft of unsoftened long bone, stained with nitrate of silver. B. (Fig. 31.)*

Under the low power, observe the peripheric lamellæ on the outside, and the perimedullary on the inside, of the shaft; and between the two forming the greater part of the bone, the

Haversian systems and intermediate lamellæ—the latter formed of the remains of previous peripheric and Haversian lamellæ. *Fig. 31* shows only the Haversian and intermediate lamellæ. The nitrate of silver has stained the more recently laid down tissue more deeply than the older parts, so that the outlines of the Haversian systems are better defined than in an unstained specimen. The origin of some of the intermediate lamellæ is well shown in the several older, half absorbed, Haversian systems. Under the high power, study the parallel arrangement of the lamellæ in the different systems, and the position and shape of the lacunæ and canaliculi. Observe the free anastomosis between the canaliculi of neighbouring lacunæ. Observe the canaliculi opening into the Haversian canals, and those at the periphery of the systems—the recurrent canaliculi—bending back to anastomose with each other. As a rule, these canaliculi of the peripheral lacunæ do not anastomose with those of adjacent systems.

(2,) *L. S. Shaft of unsoftened long bone. (Fig. 34.)*

Here the Haversian canals may be seen, running for the most part longitudinally, communicating with each other by transverse or oblique branches, and opening on the one hand into the medullary cavity, and on the other on to the surface of the bone. Observe the lamellæ seen in section, running parallel to the canals they surround, and the flattened lacunæ (fusiform in section) with their long axis in the same plane. As before, under the high power, observe the relations of the lacunæ and canaliculi.

(3,) *V. S. Frontal or parietal bone unsoftened. (Fig. 33.)*

Examine under the low power, and identify on either side of the specimen, the tables of the skull (*a*) formed of compact bone, traversed by Haversian canals, seen in transverse, oblique, or longitudinal section; and the diploë of cancellous bone between them (*b*). Observe the difference between the tables and the diploë. It is merely a question of the relative amount of bone and the size of the spaces; in the tables the amount of bone is great proportionately, and the spaces (the Haversian canals) small; in the diploë the bone is greatly diminished in quantity, and the spaces (the cancelli) are very large, thus giving it a spongy character. To the naked eye, the line between the compact and spongy bone in this situation is more or less abrupt, but under the microscope, the transition appears more gradual.

(4.) *T. S. Shaft of softened adult long bone of cat, stained with picro-carmin.* F.

Under the low power, observe the broad ring of compact bone, becoming cancellous at its inner surface where it surrounds the medullary cavity. The latter is usually empty, the contents having dropped out in the course of preparation. Note the two layers of the periosteum surrounding the bone—an outer, dense fibrous layer stained deeply with the carmine, and an inner, more delicate one, forming a light band between the outer and the bone. Put on the high power over the periosteum; examine the deeper layer, and observe that though it is much more delicate and cellular than the outer, fibrous one, the cells have not here the distinctive characteristics of the osteoblasts to be seen in a section of growing bone in which the osteogenetic layer is in a state of functional activity. Look round the specimen, and observe if the insertion of a tendon is to be seen at any point of the circumference; if so, the periosteum will be observed to be thickened at the spot, and the strands of tendinous fibres passing more or less obliquely through both layers, may be seen piercing the bone itself and becoming lost in its substance. The tendon is in fact continuous with the periosteum, and the fibres piercing the bone are of the same nature as Sharpey's fibres, of which they form a special collection. Examine the bone itself, and note that in this softened specimen, the lacunæ and canaliculi are no longer to be seen standing out clearly as when they were filled with air. The contained bone corpuscle, stained yellow with the picric acid, is alone visible as a somewhat stellate or irregular cell, the fine processes of which are not readily seen. Note the Haversian canals in transverse section, no longer appearing black, but containing blood-vessels, etc., supported by a little delicate connective tissue.

(5.) *Red marrow of rabbit, in normal saline.*

Extract some of the red marrow from the broken end of a rib or long bone of a rabbit. Diffuse on a slide with a needle in a drop of normal saline; cover; examine with the high power—preferably, one magnifying at least 600. Look for the different kinds of marrow cells; examine the small cells especially, noting that the nucleus is not at first clearly visible. By the method of irrigation introduce a drop of acetic acid, when it becomes distinctly revealed. (Not permanent.)

FIG. 37.

V.S. CRANIAL BONE OF FŒTAL MOUSE (INTRA-MEMBRANOUS OSSIFICATION), STAINED WITH BORAX-CARMINE $\times 300$.

- a.*—Fibrous layer of periosteum.
- b.*—Growing osteogenetic layer of periosteum.
- c.*—Calcified fibrous tissue.
- d.*—Osteoblasts.
- e.*—Capillary blood-vessels.

FIG. 38.

L.S. HEAD OF DEVELOPING LONG BONE, STAINED WITH
HÆMATOXYLIN $\times 40$.

- a.*—Cartilage of head of bone.
- b.*—Cartilage cells arranged in rows.
- c.*—Calcified cartilage trabeculæ with bone laid down on them.
- d.*—Periosteum.
- e.*—Termination of periosteum, fusing with hyaline cartilage of head of bone.

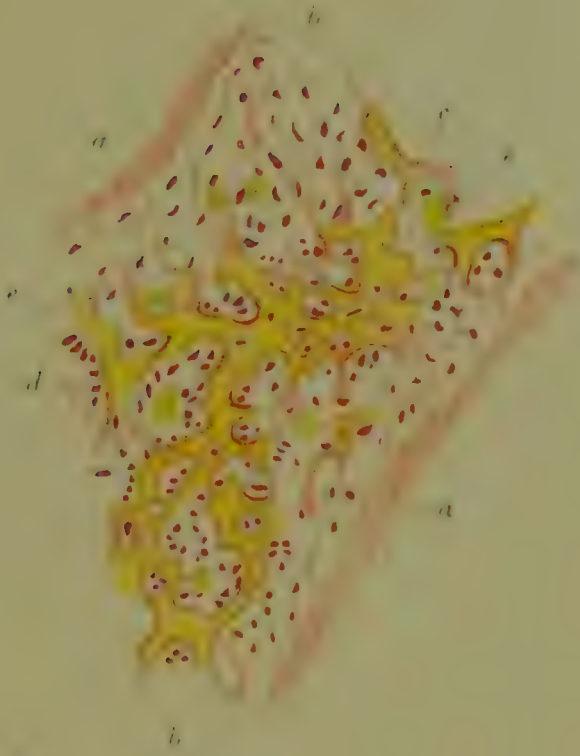


Fig. 38.



(6,) *Red marrow of rabbit, stained with picro-carmin.* G. J.

Shake up a portion of red marrow in Hayem's fluid in a test-tube, set aside and allow to stand for twenty-four hours; decant, wash, stain in picro-carmin for twenty-four hours; decant, cover with melted glycerine jelly; stir up, to diffuse the sediment evenly through the medium; cool rapidly under the tap so that the marrow has not time to settle at the bottom again. Mount a drop in the ordinary way. Examine under the high power.

(7,) *Cover glass preparation of red marrow of rabbit, stained with methyl-blue.* B. (Fig. 35.)

Squeeze a little of the red marrow between two covers, slide them from each other, dry, stain with methyl-blue, and mount in balsam. Examine under the high power.

DEVELOPMENT OF BONE.

(8,) *T. S. Cartilaginous bone of fœtal cat (stage of proliferation and calcification), stained with hæmatoxylin.* B.

Under the low power, observe in the centre of the cartilaginous shaft, a more deeply stained area, in which the cartilage cells, which have increased in size and proliferated, are separated from each other by trabeculæ of matrix which has become calcified and is stained deeply with the reagent. Under the high power, examine this area more carefully. In many cases, several cartilage cells are massed together in one cavity (primary areolar space). Look for vertical rows of cartilage cells, extending from the centre toward the ends of the bone; for any indication of the irruption of vessels from the periosteum towards the calcified area; and also note, if a thin layer of bone, deeply stained with the hæmatoxylin, is beginning to appear beneath the periosteum.

As will be readily understood, the appearances vary very much according to the progress of ossification, and the specimen may accordingly show a little less or more than the above.

(9,) *L. S. Phalanx of human fœtus, stained with hæmatoxylin.* B. (Fig. 36 B.)

The human phalanges are very suitable for showing the sheath of periosteal bone. Their small size, too, readily enables one to mount the whole bone, which is of advantage in examining with the low power. For the high power, it is often more satisfactory to take a smaller portion of a bone of larger size from the cat

or rabbit, and make the thinness of the section the object to be attained rather than its completeness.

Under the low power, observe the cartilaginous heads of the phalanx stained a light blue, dotted over with cartilage cells, which become arranged in vertical rows (*c*) as the line of progression of the ossific process is approached. At this line, where the cartilage in a solid form ends, note the fringe of deeply stained processes projecting towards the centre of the shaft. These are the cartilaginous trabeculæ, and the capillary loops between them may often be observed as yellow lines, running up from the marrow which fills the central part of the shaft. Now look for the periosteal sheath of bone (*a*), surrounding the central cavity. It is deeply stained, thicker about the centre of the shaft, and thinning away as it approaches the ends, where it fades away about the level of the line of progression. Also trace the periosteum covering it towards the ends of the bone, and note that at about the same point or a little beyond, it fuses with the cartilaginous head of the bone. Examine the central medullary cavity, and observe that nearly all the cartilaginous bone has been removed, only the periosteal sheath being left. In passing away from the centre towards the ends cartilaginous bone, continuous with the fringe of calcified cartilage trabeculæ mentioned above, is again met with. Now examine with the high power the contents of the medullary cavity—the marrow. This is an example of red marrow, as at this stage of development the formation of fat cells has not taken place to any great extent. The marrow cells are so heaped up together that their characters are not so obvious as in the previous specimens, and the venous sinuses should be more especially looked for. Observe the large size of the channels, the fact that they inter-communicate with each other, and the thinness of their walls. Here and there osteoclasts may be seen removing any remnants of cartilaginous bone.

Fig. 36 A shows the stage of development diagrammatically. Observe in it the calcified cartilage matrix (*a*) stained blue, the bone covering it (*d*) stained red, in thicker layers at the centre of the shaft, and decreasing in thickness as it is traced towards either end; the periosteal sheath of bone (*c*) lying between the endochondral, and the periosteum (*b*). Note that this contains no calcified cartilage, that it is much denser than the endochon-

dral bone, that it is thicker towards its middle part, and thins away towards either end. Trace the periosteum into the cartilaginous ends of the bone, and note the vertical rows of cartilage cells (*e*). In the figure, the centre of the shaft is shown still occupied by endochondral bone, but very frequently it has been removed to a great extent, at this stage, to allow of the formation of the medullary cavity.

(10.) *L.S. Head of tibia of fetal sheep, stained with hæmatoxylin. F. or B. (Fig. 38); or with picro-carmin. F. (Fig. 39.)*

This specimen only shows a portion of developing bone, the line of progression of the blood-vessels towards the cartilaginous extremity, and is intended mainly for examination under the high power. Before mounting the specimen, observe it as it lies in the water. The greater part consists of the cartilaginous head, and will vary in shape according to the bone from which it was obtained. There is no difficulty in recognising the shape of the head of such bones as the femur and tibia. Determine the articular surface, and note the side furthest away from it. It usually has a more or less straight border, from which a delicate fringe may be seen projecting, with a stronger looking tag at each extremity of the line. The line is that of progression of the blood-vessels, marking out the limit of the ossific process. The fringe is composed of calcified cartilage trabeculæ, and the tags at each end are the periosteum, sometimes with a layer of periosteal bone beneath it. Draw the section into the requisite position on the slide, by fixing its articular edge, taking care that the parts referred to do not become folded over each other, or otherwise displaced or injured. Stain one specimen with hæmatoxylin and mount in balsam, another with picro-carmin, and mount in Farrant's solution.

Examine the first under a low power (*Fig. 38*). Place the specimen under the microscope, so that, on looking through the tube, the fringe of cartilage trabeculæ will be towards, and the articular surface away from, you. Observe the line of progression of the blood-vessels—the area already invaded by them is marked off sharply from the cartilaginous head by the yellow or green colour of the red corpuscles which have not taken in the hæmatoxylin stain. Note the deeply stained cartilage of the free trabeculæ (*c*), and that this deeper staining, indicative of calcification, is prolonged upwards into the still solid cartilage between

FIG. 39.

L.S. HEAD OF HUMAN FŒTAL TIBIA SHOWING "LINE OF PROGRESSION,"
STAINED WITH Picro-Carmine $\times 350$.

- a.*—Rows of cartilage cells.
- b.*—Enlarged cartilage cell.
- c.*—Calcified cartilage.
- d.*—Bone laid down on calcified cartilage trabeculæ.
- e.*—Osteoblasts which have become bone corpuscles.
- f.*—Osteoclasts.
- g.*—Capillary blood-vessels.

FIG. 40.

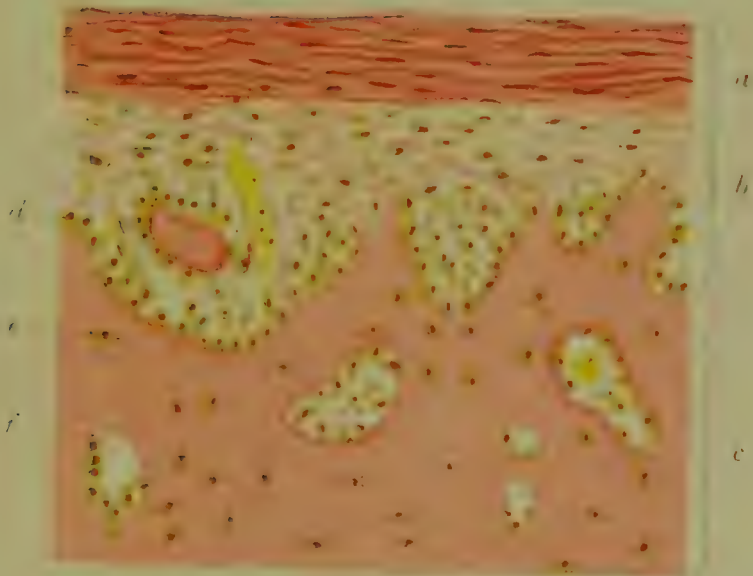
T.S. DEVELOPING BONE OF KITTEN, TO SHOW PERIOSTEAL BONE
FORMATION, STAINED WITH Picro-Carmine $\times 300$.

- a.*—Outer fibrous layer of periosteum.
- b.*—Inner cellular „ „ „
- c.*—Bone.
- d.*—Layer of osteoblasts.
- e.*—Bone corpuscle.
- f.*—Haversian space.

Fig. 39.



Fig. 40



the vertical rows of cells (*b*). At (*e*) observe the fusion between the periosteum (*d*) and the cartilage (*a*).

Examine the picro-carminic stained specimen (*Fig. 39*) under the high power for the more minute details. Find the line of progression as before. Study the cartilage cells in vertical rows (*b*), and note that each cell is in its own cavity. Observe the cartilaginous trabeculæ (*c*), and the bone laid down on them (*d*). It is very thin immediately below their point of attachment to the cartilage, but gradually increases in thickness as it is traced downwards towards the centre of the shaft. Note the scalloped outlines of the remains of the cartilage trabeculæ; the osteoblasts, as shown in the figure, often forming an almost continuous layer on their surface, sometimes as seen at (*e*) included in the semi-lunes of bone filling the concavities in their outline, to remain as bone corpuscles; the capillary loops (*g*); and the multi-nucleated osteoclasts (*f*). These can usually be found without difficulty, attached to the ends or sides of the trabeculæ as seen in the figure. Examine carefully in this specimen the gradual transition between the connective tissue cells of the periosteum at the point where it joins the cartilage and the cartilage cells. Observe that the fibres of the periosteum gradually become lost in the cartilage. Examine the osteoblasts forming a continuous layer in the deeper part of the periosteum, and note if any bone has yet been formed beneath it at this level.

(11.) *T.S. Bone of fetal kitten, stained with hæmatoxylin and carmine, B. (Fig. 41), and with picro-carminic F. (Figs. 40 and 42.)*

It is important that the transverse section should show both the endochondral and periosteal bone, so that the two may be contrasted. It should be taken, therefore, not through the centre of the shaft, because the cartilaginous bone is first removed from there, but nearer to the line of progression of the blood-vessels. In the first specimen (*Fig. 41*), observe under the low power the periosteum (*a*), with the layer of osteoblasts in its deeper, more lightly stained osteo-genetic layer; the periosteal bone (*b*), with its Haversian spaces not yet converted by deposition from within into Haversian canals; the much more spongy, open, endochondral bone (*c*). Note that the endochondral and periosteal bone are sharply separated from each other, not only by their relative density, but by the presence in the latter of the remains of the calcified cartilage matrix, in this case stained blue (*g*). Observe

FIG. 41.

T.S. DEVELOPING LONG BONE OF KITTEN (SEMI-DIAGRAMMATIC) TO SHOW ENDOCHONDRAL AND PERIOSTEAL BONE, STAINED WITH CARMINE AND HÆMATOXYLIN $\times 100$.

- a.*—Periosteum.
- b.*—Periosteal bone.
- c.*—Endochondral bone.
- d.*—Osteoblast (bone corpuscle), surrounded with recently laid down bone.
- e.*—Osteoblasts as yet unsurrounded with bone.
- f.*—Blood-vessel.
- g.*—Calcified cartilage matrix.

FIG. 42.

T.S. DEVELOPING LONG BONE OF KITTEN, STAINED WITH Picro-CARMINE $\times 400$.

- a.*—Periosteal bone.
- b.*—Endochondral bone.
- c.*—Remains of calcified cartilage matrix.
- d.*—Bone laid down in form of lunules.
- e.*—Osteoclast.
- f.*—Osteoblasts.
- g.*—Bone corpuscles.

Fig. 41.

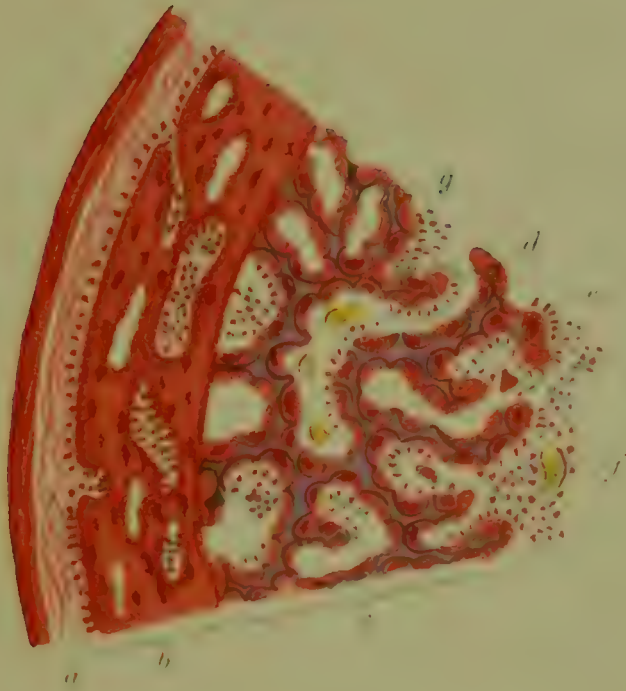
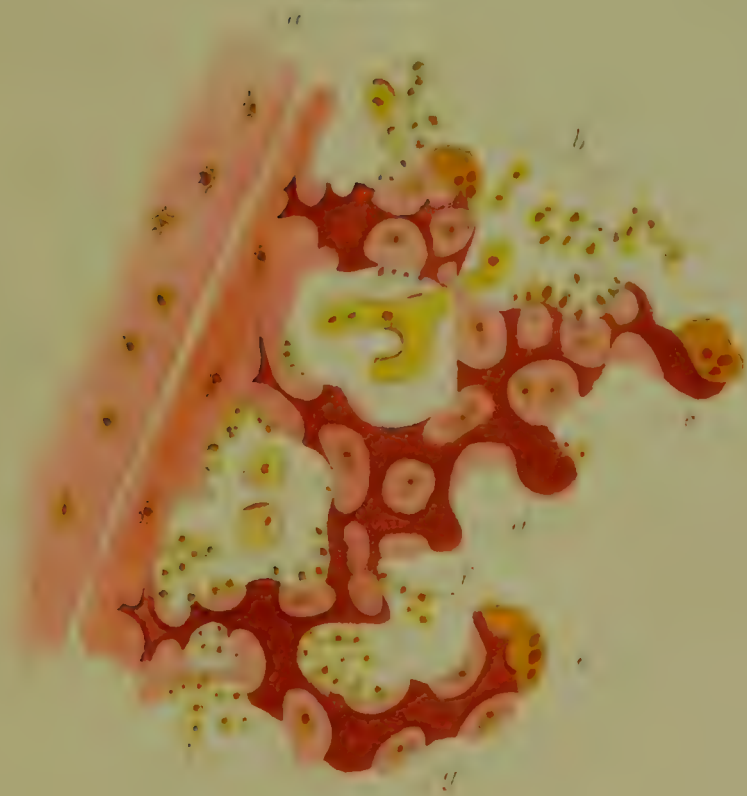


Fig. 42.



the spaces in the endochondral bone, filled with osteoblasts (*e*) and capillary blood-vessels (*f*).

Examine the picro-carmin stained specimen under the high power, after noting again the general arrangement of parts with the low. Examine the two layers of the periosteum (*a*) and (*b*) (*Fig. 40*), and note the layer of osteoblasts (*d*) in the deeper part of (*b*) in close contact with the bone (*c*). Observe their somewhat columnar shape, and that they frequently exhibit processes on the side in contact with the bone. As they become submerged in the bone secreted around them, processes are given off from their general periphery, and they assume the characters of bone corpuscles. Observe carefully the projections of bone into the periosteum. It is often possible to see the appearance, represented in the figure, of the fibres of the periosteum being included at the summit of these processes. Note the spaces beneath the surface of the bone lined by osteoblasts. Observe the branched bone corpuscles lying in their lacunæ. Find the junction of the periosteal (*a*), with the endochondral bone (*b*) (*Fig. 42*), and identify the appearances shown. Note the remains of the calcified cartilage matrix (*c*), and observe the scalloped outlines before referred to. In the bays observe the semilunes of bony tissue, each containing a bone corpuscle (*g*). Study the contents of the areolar spaces—the layer of osteoblasts, frequently columnar in shape, and placed vertically, lining them (*f*), the sections of capillary vessels, and the multi-nucleated osteoclasts (*e*).

(12.) *V.T.S. Head of fatal mouse for membrane bone, stained with borax-carmin. B. (Fig. 37.)*

This section should be cut in paraffin, as the parts readily fall from each other when placed in water. Under the low power, identify the general arrangement of parts, which varies with the position, from before backwards, at which the section was taken. The central hemispheres, the eyes, rudimentary teeth, and olfactory membrane may be seen.

The tongue, cut in transverse vertical section with the mouth cavity above it, is always visible unless the section has been taken too far back. Development of bone in membrane is very readily studied in this specimen. Look first at the section of the lower jaw on either side of the tongue, and then at the membrane bone in other parts of the head. Select a portion of one of the flat bones (*Fig. 37*) and put on the high power. Note, as shown

in the drawing, the periosteum on either side, with its two layers, fibrous (*a*), and cellular (*b*). Between the cellular layers note the fibrous membrane undergoing ossification. Observe the nature of the change which is taking place in it. The strands of white fibrous tissue, especially about its centre, are becoming calcified (*c*), and highly refractile in consequence. The cells between these fibres are beginning to secrete bone, and are becoming included as bone corpuscles (*d*). The drawing is a camera lucida one, and accurately represents a very usual appearance of developing bone in membrane; it may serve as an illustration to the student of the fact that he must not look for diagrams under the microscope. One specimen will show one point well—in this case the calcification of the fibres—another may bring out something else, perhaps the vascular network, a third some other point, so that the full mental picture of the structure frequently requires to be built up of several sections. The failure to recognise this on the part of the student is apt at first to cause him trouble with his specimens. He expects to find in them all the features of a diagram, and is consequently usually subjected to disappointment.

Now examine the section of the lower jaw. In the centre look for Meckel's cartilage, itself cut transversely. It is almost entirely cellular at this stage of development, with very little hyaline matrix. Around it, note the membrane being converted to bone, the process being initiated by the calcification of the fibrous tissue. Note as before, the peculiar refractile appearance of the fibres. Trace them outwards, and observe that they are continuous with the uncalcified soft fibres at the periphery of the area.

The specimen is often valuable for other things to be seen in it, especially for olfactory mucous membrane and developing tooth.

APPENDIX TO CHAPTER VI.

1. Sections of unsoftened bone, stained with Silver Nitrate.—Unstained sections are better bought already mounted. If, however, it be desired to stain with nitrate of silver, the student will require to prepare it himself as follows : Select a bone, such as the radius or humerus, which has been macerated and cleaned in the ordinary way for the study of anatomy. It is better that it should not have had the organic matter too thoroughly removed ; it should be fairly heavy and solid, rather than light and dry. Saw it into two parts, and with as fine a saw as obtainable ; remove a thin slice from one of the cut ends. It is not necessary to take a slice of the whole shaft in transverse section. The piece has next to be ground down to the requisite thinness, and stained with silver nitrate ; and the two processes can be carried on simultaneously. It is well to use two stones, a rough and a smooth one ; the rough, in order to get the section rapidly reduced in thickness, the smooth, to complete the process more carefully. Pour a few drops of 1 per cent. solution of silver nitrate on the first stone, and commence to wear down the bone by moving it rapidly beneath the tip of the forefinger from side to side, or in circles upon the surface. This is continued for some little time ; the piece of bone is then turned with its other side to the stone, and the process repeated. When the requisite thinness is attained, the wearing down is carried a little further, still in the silver solution, upon the second stone.

If it be preferred a flat piece of cork may be interposed between the finger and the section, which moves with the cork and upon the stone. There is less likelihood, in this way, of the specimen being broken when it is beginning to get really thin. It may now be thoroughly washed and dehydrated in absolute alcohol, cleared with clove oil, and mounted in balsam. The dehydration and clearing both require to be done in watch glasses. As in other cases of silver nitrate staining, there is a little uncertainty in the result, but when successful it is very beautiful. It is not advisable, as before stated, for the bone to be too dry and light—in that case there appears to be not enough organic matter left to cause the reduction of the silver salt.

2. T. Section of long bone of adult cat.—Decalcify part of the femur of a full-grown cat in chromic and nitric fluid ; harden in Müller and spirit ; cut in gum.

3. Developing bone, stage of proliferation and calcification.—Take one of the bones of a foetal kitten which will cut longitudinally with a knife without previous decalcification. The section should show a darker area in the centre of the shaft, and perhaps a little grittiness may have been noticed

as this part was reached in cutting. If the grittiness was very distinct, decalcification may be advisable for a day or so, in chromic and nitric fluid. Harden in Müller and spirit; cut in gum.

4. **Developing bone, stage of bone formation.** — Take one of the long bones, *e.g.*, the femur of a new-born kitten, and test the stage of ossification with the scalpel or needle. It should resist the passage of either to an appreciable extent. Soften in chromic and nitric fluid; harden in Müller and spirit. Use the head of the bone for longitudinal sections, and take a piece of the shaft not far from the head, for transverse. It is better to avoid the mid shaft, as the endochondral bone has probably been, at this stage, removed. For transverse sections, the bone can be stained in borax-carminé, and cut in paraffin with advantage, as otherwise the endochondral bone has a great tendency to fall out of position. It is well to put up a variety of bones instead of one, there being thus greater certainty of obtaining the stage of development required.

5. **V.S. Membrane bone.** — The head of a foetal animal, such as the mouse is very serviceable. Decalcify, if necessary, in chromic and nitric fluid; harden in Müller and spirit, stain in borax-carminé, cut in paraffin, and mount in balsam. The bones of the vault of the skull of any small foetal animal will do, however, provided ossification has not advanced far.

CHAPTER VII.

*THE SIMPLE TISSUES (Continued).**MUSCLE AND NERVE TISSUES.***MUSCULAR TISSUE.**

MUSCULAR tissue is composed of muscle fibres or cells, arranged in a fascicular manner, and embedded in ordinary connective tissue containing blood-vessels, lymphatics, and nerves. Muscular fibres are striated or non-striated. The skeletal muscles, by which ordinary voluntary movements of the body are performed, are striated and unbranched; the heart is composed of striated branched fibres; the muscular walls of the hollow viscera and the blood-vessels of non-striated fibres. It will thus be seen that, with the exception of the heart, striated muscle is under the control of the will, or is voluntary, as it is termed. Non-striated muscle, on the other hand, with exceptions afterwards mentioned, is involuntary.

THE STRUCTURE OF ORDINARY SKELETAL MUSCLE.

This may be conveniently studied under the following five headings:—

(1.) *The shape and size of the cells.*—They are cylindrical in form, tapering to a blunted point at their extremities. In size a cell is $\frac{1}{1000}$ into $\frac{1}{30}$ inches in breadth, and 1 to $1\frac{1}{2}$ inches in length, but both the breadth and length may vary considerably.

(2.) *The presence or absence of a separable envelope.*—Each cell is enclosed in a delicate, elastic, homogeneous, translucent envelope—the sarcolemma—which is adherent to the cell contents during life, but can readily be separated after death.

(3.) *The number and position of the nuclei.*—Each cell possesses many nuclei, and in mammals these are placed immediately

FIG. 43.

NON-STRIPED MUSCLE CELLS, STAINED WITH HÆMATOXYLIN.

- a.*—Dissociated cells from intestine of cat $\times 300$.
- b.*—T.S. Muscle cells of external muscular coat of intestine of cat $\times 300$.
- c.*—Middle part of isolated cell, under a higher power.

FIG. 44.

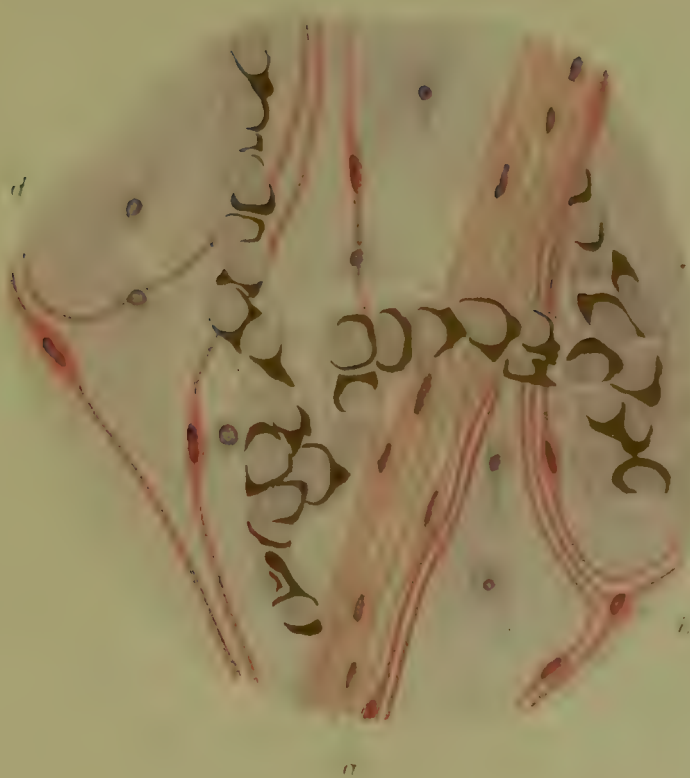
BLADDER OF SALAMANDER, STAINED WITH HÆMATOXYLIN AND
EOSIN $\times 300$.

- a.*—Non-striped muscle cells.
- b.*—Tri-radiate cell.
- c.*—Blood capillary containing altered corpuscles.
- d.*—Connective tissue cell of bladder wall.

Fig. 43.



Fig. 44.



beneath the sarcolemma. They are oval or elongated in shape, placed in the long axis of the cell, and frequently surrounded with a little undifferentiated protoplasm. In the frog they are not confined to the above position, but are scattered throughout the cell contents. In the wing muscles of some insects they are found in linear series in the central axis of the cell.

(4.) *The relation of the cells to each other and the connective tissue which supports them.*—The cells lie parallel to each other in bundles, which are collected again into larger fasciculi. The whole muscle is invested with a sheath of ordinary connective tissue, the *epimysium*; which sends inwards septa, splitting up the mass of muscle fibres into larger and then smaller bundles, the *perimysium*; and from the *perimysium* still smaller prolongations of delicate tissue, the *endomysium*, penetrate between the individual fibres themselves. At either end of the muscle the connective tissue of the epi-, peri-, and endomysium becomes condensed as the muscle fibres themselves become fewer, and finally merges in the tendon of the muscle, with which it is continuous. Where the tendon and muscle join the connective tissue, fibrils of the former become closely united to the conical ends of the sarcolemma of the muscle cells, but not to the cell substance itself. Throughout the course of the muscle, the cells are united in the same manner to the connective tissue by which they are surrounded by their pointed extremities, and as this is in continuity with the tendon at either end, each individual muscle cell acts more or less directly upon the insertion and origin of the muscle. The connective tissue is freely supplied with blood-vessels. The larger branches run in the epi- and perimysium, and terminate in capillaries, which enter the bundles in the endomysium, and form a network with elongated meshes between the muscle fibres themselves. The longer strands of the capillary network run in a direction parallel with the fibres, and communicate with each other by transversely running loops. The connective tissue is permeated with a lymphatic system originating in the cell spaces, so that the muscle cells are bathed in lymph, which lies, however, external to the sarcolemma. The nerve terminations of muscle are peculiar, and will be considered later. It may be stated, however, that each cell possesses about its middle, immediately beneath the sarcolemma, an oval end plate in which a medullated nerve fibre terminates.

(5.) *The structure of the cell substance.*—When a muscle cell is

viewed longitudinally it is seen to be transversely striated, and, to a less extent, also in its long axis. It is from the former that its name, "striated," is derived. These transverse markings consist of alternate light and dim lines, which vary in relative breadth with the state of extension or contraction of the muscle. By means of reagents it is possible to cause a cell to split transversely into discs, the cleavage being due to softening of its structure in the region of the light stripe. The longitudinal striation of the fibre is due to the fact that the cell substance within the sarcolemma is arranged in the form of a number of fibrillæ of peculiar structure, cemented together. The fibrillæ can be separated readily from each other by teasing, especially in the muscle fibres of a crab's claw which has been preserved in alcohol. They vary very much in breadth in different animals, but are necessarily extremely narrow compared with the cell of which they form a part. If a transverse section of a muscle fibre be examined under a high power it will be seen to be marked out into a number of somewhat polygonal areas, bounded by light lines. The areas are small bundles of fibrils, and the intervening network of light lines the cement substance lying between them, which also penetrates between the fibrillæ themselves. This network stains deeply with chloride of gold, and may provisionally be regarded as a less differentiated form of the original cell protoplasm. It differs in quantity in the muscles of different animals. In the wing muscles of some insects, it is very large in amount, and forms a well marked ground substance in which the fibrils are embedded. In the more highly developed amphibian muscle it is much smaller in quantity, the greater part of the fibre having, in this case, undergone differentiation to form contractile fibrils. This substance between the fibrils was named by Rollett the "sarcoplasm."

When a small piece of muscle has been suitably prepared, stained, and teased, the fibrils may be seen to have very distinctive characters. Each consists of an alternation of light and dark segments, by the lateral apposition of which, the light and dark stripes of the uninjured fibre result. The light segment is, to a great extent, unstained by the reagent, while the dark is deeply affected. Compared with the width of the fibril, the segments are long, but their length, both relatively to each other and also absolutely, varies considerably with the contraction or relaxation of the muscle. In a fibril which has been stretched

to its full physiological extent, the light is slightly longer than the dark segment, and has the following characteristics :—

In its centre is to be observed a round body—Dobie's globule—which stains deeply with the reagent in the same way that the dark segment does (*Fig. 53*). This globule occupies a portion of the light segment, which is also stainable, though to a less extent, which may be termed the "intermediate" segment (*A, c*), the part extending on each side of the globule (*e*) being the "nebenscheibe" of Merkel. Between the intermediate segment and the dark segment (or long sarcous element as it is also called) is an unstained portion, in which Flögel's granule (*d*) (a smaller one than Dobie's, but staining in the same way) occurs. The dim, or dark segment, or long sarcous element, stains deeply, like Dobie's globule and Flögel's granule, with reagents such as hæmatoxylin, eosin, or aniline-blue. It is somewhat of the shape of a dumb-bell, but this is better seen when the fibril is contracted. In the extended condition (*A, a*) the segment appears cylindrical, with a slight bulging at either extremity where it adjoins the light segment, and another less marked in its centre, which gives rise in the contracted state to Hensen's line (*F, f*).

If a part of a cell be examined in which the fibrils have not been separated from each other, the following appearances may be noted as the result of the fibrillar structure described above. The long sarcous elements of adjacent fibrils, being placed side by side palisade-wise in the same plane, give rise to the dim stripe seen on the whole fibre. Between these the laterally opposed light segments are placed, and give rise to the light stripe.

Both these stripes, light and dark, when seen in the fibre are narrow, with their length running across the fibre; when seen in the fibril, they are still narrow, but in their other diameter; their length running with the axis of the fibril. This is easily to be understood by comparison with a palisade. The length of the palisade runs parallel with the ground; the length of the stakes forming it is vertical to the ground. The stripes, as seen crossing a fibre, correspond with the palisade as a whole; the segments, as seen in the fibrils, to the individual stakes.

If the light stripe be carefully examined, it will be seen to be traversed in its centre by a dotted line, caused by Dobie's globules in lateral series; and, if the power used be high enough to reveal them, both the "intermediate segment" and Flögel's

FIG. 45.

STRIATED MUSCLE OF FROG, TEASED $\times 250$.

- A.—Muscle fibre, stained with picro-carmin.
- B.— " " ruptured, showing twisted sarcolemma.
- C.— " " after treatment with ammonium carbonate, showing
transverse cleavage into discs.

FIG. 46.

TENDON AND MUSCLE FIBRES FROM WING OF COMMON HOUSE-FLY,
STAINED WITH PICO-CARMINE $\times 200$.

- a.*—Tendon.
- b.*—Muscle fibres.

FIG. 47.

SECTION OF STRIPED MUSCLE, INJECTED, FROM TONGUE OF CAT
 $\times 250$.

- a.*—Small artery or arteriole.
- b.*—Capillary.
- c, c'.*—Vessels in deeper plane of section.
- d.*—Muscular fibre.

Fig. 45.



Fig. 46.

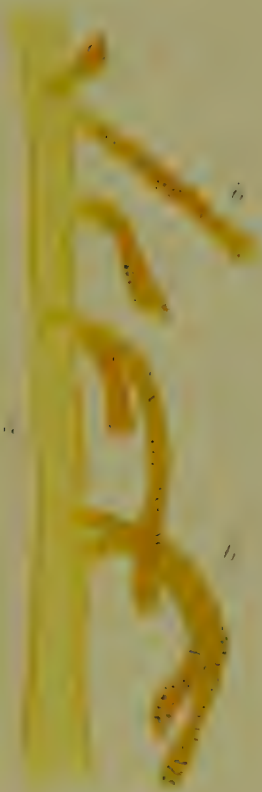
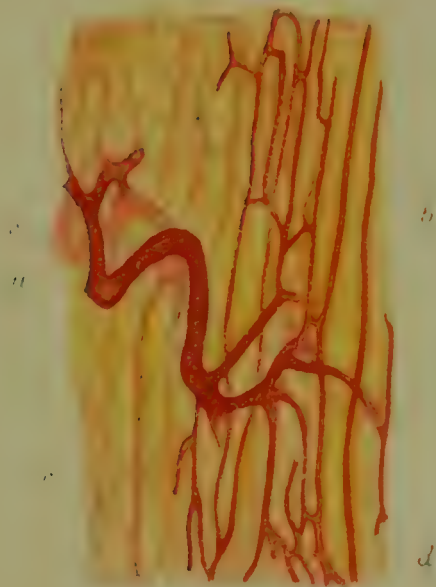


Fig. 47.



granules may be made out, but the lens used must be considerably stronger than that employed for ordinary purposes. An oil immersion lens, $\frac{1}{2}$ or $\frac{1}{8}$ inch, and a sub-stage condenser, are usually required to enable one to observe the structure of the light segment in extended muscle of crab, as described above. In using a lens, magnifying about 400 or 500 diameters, it will only be possible to distinguish the alternate light and dark stripes, with the line crossing the former, due to the lateral apposition of Dobie's globule.

Nature of the Fibrillar Structure.—The ultimate structure of the fibrils is still very doubtful. It has always been a subject of great interest to histologists, but it is not possible as yet to give any theory which may be considered to be generally accepted. The differentiated protoplasm of the fibril has, no doubt, a complex structure. The outer part is possibly in the form of a membrane, enclosing merely a fluid where it surrounds the clear segment; but in addition, a reticular basis, the meshes of which are occupied by a myeloid substance, staining with certain reagents more or less deeply, where it forms the wall of the long sarcous and intermediate segments.

But though the structure of the muscular fibril cannot be regarded as definitely determined, the following changes, which take place during the contraction of the fibre, can (whatever the true interpretation to be placed upon them) be followed with a sufficiently high power without much difficulty in a specimen of crab's muscle, stained with eosin or hæmatoxylin. The student is referred to *Figs. 51* and *53*, in which the fibrils are shown in different stages of extension and contraction.*

Figs. 51 and *53 A* both show an extended fibre as already described. It will be seen that the long sarcous element (*Figs. 51 B* and *53 A a*) is, in this condition, about the same length or a little shorter than the light segment (*Figs. 51 A* and *53 A b*). Observe the intermediate segment (*Figs. 51 C* and *53 A c*) in the middle of the light, and note that its shaft is not clubbed at the end, in this way differing from the long sarcous element. It is also much less deeply stained, from the fact, perhaps, that it contains less of the myeloid substance to which the staining is due. In fact,

* This account of the changes observable in contracting muscle is virtually that given by Professor Rutherford, of Edinburgh, to whom the author acknowledges his indebtedness.

this substance may be entirely absent from the segment, and it is then not seen as distinct from the rest of the clear stripe.

In both the figures, however, it is shown as it is usually to be seen, lightly stained, and on either side of it the clear segment, with Flögel's granule in it (*Figs. 51 c and 53 A d*). It is through the clear segment that cleavage of the fibre takes place, but as the envelope of the clear segment on either side of the break collapses, this appears to have taken place, either at the level of the ends of the long sarcous, or the intermediate segments.

Fig. 53 B shows a few fibrils entirely uncontracted, but not extended to their full physiological length. They are merely in a state of relaxation. Observe that here the clear segment has disappeared, together with Flögel's granule, the heads of the sarcous elements having come into contact with the ends of the intermediate segments. It will thus be seen that in the relaxed, as opposed to the extended fibre, the light stripe is somewhat narrower than the dark.

Fig. 53 C shows the commencement of contraction. The dark sarcous elements (*a*) have become shorter and thicker, their myeloid substance appearing to accumulate in their heads, which thus become enlarged and more deeply stained, while the centre of the shaft is left clearer. The intermediate segment is also appreciably shortened.

Fig. 53 D shows a further stage of contraction. The shortening and thickening of the dark sarcous elements (*a*) are still more pronounced, and the collection of the myeloid material in the heads, to the deprivation of the shaft, still continues. The intermediate segment is still further shortened, and Dobie's globules are now commencing to be flattened by the encroaching heads of the sarcous elements on either side. At this stage the outline of the fibre begins to show distinct bulging at the level of the heads of the sarcous elements.

Fig. 53 E, the next stage, shows the same process carried still further, the shaft of the sarcous elements being now quite light, from continued withdrawal of the myeloid substance, which has passed into the heads. In the centre of the shaft, Hensen's line is now becoming visible. Dobie's globules are still further compressed by the heads of the sarcous elements, and now form, with the heads on either side of them, the dark stripe of the contracted fibre, in which, however, the outlines of the structures forming it, can still be distinguished. Observe that there is a

distinct bulging in the contour of the fibre opposite the new dark stripe.

Fig. 53 F. This is the completely contracted fibre. The heads of the sarcous elements of adjacent rows have fused with Dobie's globules and the intermediate segment, to form a new homogeneous dim stripe, while their shafts have become clear, and constitute the new light stripe, in the middle of which Hensen's line may be clearly seen. Thus, a reversal of the stripes seems to take place during contraction; but it will be noted that the dark stripe of the contracted fibre represents something more than the light stripe of the uncontracted. It represents in addition the heads of the sarcous elements. Again, the light stripe of the contracted fibre does not represent the whole of the dark stripe of the uncontracted, but only the shafts of the long sarcous elements.

In this, the final stage of contraction, the beaded appearance of the fibres is well seen, their contour bulging opposite the new dark stripe, and showing a concavity opposite the light.

The reversal of the stripes described above must be carefully distinguished from that which depends upon the focus. This is precisely comparable with that which occurs in the focussing of a red blood corpuscle. If the focus is only very slightly altered, the long sarcous elements and Dobie's globules stand out as bright rods and globules respectively, while the rest of the light stripe appears dark. This difference is simply due to the different levels focussed, the first being the middle plane of the fibrils, the second their surface.

With regard to the many diverse opinions held as to the intimate structure of the fibrils, the student is referred to the literature on the subject. This is so extensive, and the matter is altogether so undecided, that it has not appeared advisable to give even a *résumé* of the views which have been held by different authorities. Allusion may, however, be made to a comparatively recent suggestion by Professor Haycraft, that the appearances of alternate striping are due to varicosity of the fibrils alone. Thus, a fibril with alternate swellings and constrictions upon it—a beaded fibril—would appear dark in the region of the swelling, and light in the internode at one focus, and *vice versa* at another. He supports the theory very ingeniously by the results of impressions in collodion of the fibres. These impressions, as may be seen in the photographs published with his paper, do certainly very fairly reproduce the ordinary appearance of the fibrils, and the contention apparently is, that inasmuch as this appearance can be produced merely as a result of the form of the fibril impressed upon the collodion, there is no reason to look for any structural variation in the different parts of

FIG. 48.

V.S. HEART WALL (HUMAN), STAINED WITH HÆMATOXYLIN $\times 300$.

- a.*—T.S. Cardiac muscle fibre.
- b.*—Connective tissue supporting them.
- c.*—Endocardium.

FIG. 49.

T.S. STRIPED MUSCLE (HUMAN), STAINED WITH HÆMATOXYLIN $\times 300$.

- a.*—T.S. Muscle fibre.
- b.*—Perimysium.
- c.*—Nuclei of endomysium.

Fig. 48.

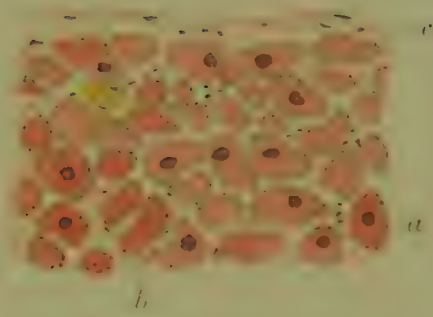


Fig. 49.



the fibril itself to account for the different appearances of different parts of it. But it is not to be lightly granted that collodion impressions produce *all* the appearances seen in the actual fibrils, and even if this were the case, it does not in any way exclude the probability of a highly complex internal structure. There does not seem to be any necessity for regarding collodion impressions of muscle fibres from a very different standpoint to that of wax impressions of naked eye objects. We are all familiar with the close resemblance between the surface of the tip of the finger and its impression upon wax; the appearance is the same, or nearly so, in each case, but whereas the wax is uniform throughout, the epithelial cells of the epidermis present a complicated structure. If a piece of cloth be pressed upon a layer of wax of suitable consistence, a fairly striking reproduction of the surface view of the fabric is reproduced, and if we previously tint our wax, the resemblance may be still further increased. But it is difficult to understand why, because we can produce a simulacrum of a muscular fibril in a homogeneous substance, we should necessarily conclude that the fibril is probably itself homogeneous and structureless. This would be tantamount to saying with regard to the skin and its wax impression, that the former was probably homogeneous and structureless, inasmuch as its image could be produced by impression on homogeneous wax. It is to be noted, too, that we can get impressions of other tissues, bone, tooth, hair, etc., which come out as well as those of muscle. Do these impressions justify us in considering that the different appearances of these tissues are merely due to external form? That, inasmuch as structureless wax can reproduce them, they are probably structureless?

In addition to the fact that the evidence of these impressions of muscle fibrils in no way excludes the likelihood or possibility of the fibrils possessing a complicated structure, the records of work by very many observers go to prove very incontestably that such a complex organization, whatever may be its precise nature, does exist; nor would we, *a priori*, be at all inclined to suppose that an organ, with so highly complex a function as that of muscle, would be so simply constructed as the above experiment might at first sight seem to imply.

The Development of Muscle cells.—These are developed from the mesoblast, in the muscle plates lying on either side of the embryonic neural column. The cells elongate and become multi-nucleated, and finally their protoplasm becomes fibrillated, the fibrillation commencing, as a rule, at one side of the cell, and spreading round it. At the same time a delicate envelope, the sarcolemma, becomes apparent. The muscle fibres are thus formed from the elongation of single cells, and not by the apposition in linear series of a number of cells, as in the case of medullated nerve fibres.

Termination of Motor Nerves in Muscle.—The nerve fibres terminate in what are termed *end-organs* or *end-plates*. The end-organ is a somewhat oval, flattened mass lying between the sarco-

lemma of the muscle fibre and the sarcous substance itself. It consists of two parts: (1,) the termination of the axis cylinder of the nerve fibre; (2,) a mass of granular material containing many clear nuclei—the *sole* or *bed* of the organ. The individual nerve fibre may reach the end-plate undivided, or it may branch previously. As it pierces the sarcolemma, the latter and the grey sheath of the fibre become continuous with each other. The rest of the fibre now usually divides into two or three primary branches, and, having done so, the medullary sheath suddenly

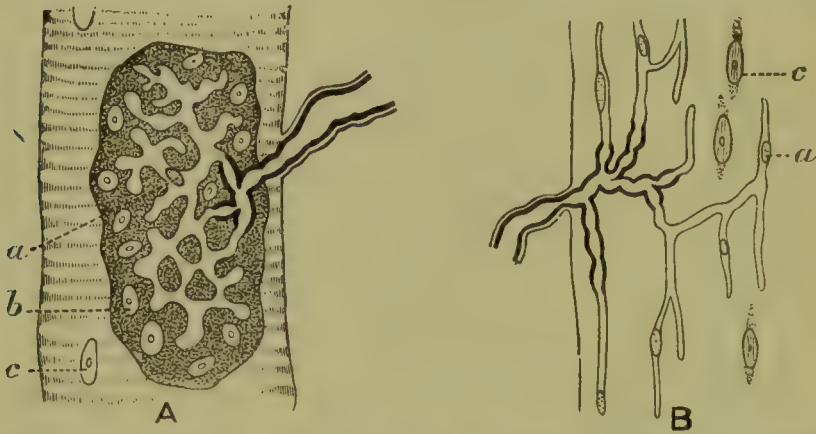


Fig. E. A.—End-organ of nerve in voluntary muscle fibre of lizard, highly magnified.

a.—Nucleus of "arborescence."

b.— " of "sole."

c.— " of muscle cell.

B.—End-organ of nerve in voluntary muscle fibre of frog.

a.—Nucleus of "arborescence."

c.— " of muscle cell.

(After Kühne.)

ceases. The axis cylinder continues to branch in a somewhat arborescent manner, the main trunks of the tree anastomosing with each other; the peripheral branches tend to terminate in somewhat thickened extremities. Here and there, upon the divisions of the axis cylinder, granular nuclei are to be seen, which are readily distinguishable from the clear nuclei embedded in the *sole*. Each muscle cell appears to have one end-plate, and, as a nerve fibre frequently branches before its termination, it follows that one fibre may supply several muscle cells.

The above is the form of end-organ to be found in mammals and some other animals. *Fig. E A* is from the muscle of the lizard. In amphibians, however, such as the frog (*Fig. E B*), the termination of the nerve fibre is not so compact, the axis cylinder branching beneath the sarcolemma, and the branches passing for some distance in the axis of the fibre, to terminate in

rounded extremities. There is no granular sole or bed. Granular nuclei are found here and there upon the course of the divisions of the axis cylinder. They are small and readily distinguishable from those of the muscle fibre itself lying beneath the sarcolemma.

THE STRUCTURE OF CARDIAC MUSCLE.

A cardiac muscle cell differs markedly from that of ordinary striated muscle. The structure may with advantage be considered under the same headings.

(1,) It is cylindrical in shape, usually branched towards one end. Seen in longitudinal section, it is roughly rectangular, and round, or sometimes oval in transverse. The ends of the cell are serrated. In breadth they are smaller than the fibres of skeletal muscle, and in length still more decidedly so.

(2,) There is a delicate ordinary envelope to the cell, but no separable sarcolemma.

(3,) Each cell possesses one oval nucleus, situated in its centre. Granules of pigment are apt to appear in the protoplasm at the poles of the nucleus, especially in old people.

(4,) The cells are joined together directly by their serrated ends, the branch of one cell anastomosing with that given off from another. Through this anastomosis a very characteristic network results. There is a little delicate connective tissue (continuous with that of the peri- and endocardium) between the muscle fibres, supporting capillary blood and lymphatic vessels. These latter are very numerous. In the case of ordinary striated muscle, on the other hand, lymph vessels appear only to be found in the epi- and perimysium, not between the individual fibres themselves.

(5,) The cell substance is striated both transversely and longitudinally, but much less distinctly than in the case of skeletal muscle. It cannot be teased into fibrils in the same manner. This is accounted for by the fact that the differentiation into fibrils is only to be seen on the surface, and does not extend throughout the cell. In transverse sections of the fibres the longitudinal cleavage may be seen to be commencing at the periphery, but to extend no further.

Purkinje's cells may be seen situated immediately beneath the lining membrane of the ventricles. They are to be found in

FIG. 50.

STRIPED MUSCLE OF CRAB (RELAXED), STAINED WITH HÆMATOXYLIN,
TEASED $\times 250$.

- a.*—Muscular fibre, or portion of one.
b.—Fibrils.

FIG. 51.

STRIPED MUSCLE OF CRAB (EXTENDED), STAINED WITH HÆMATOXYLIN,
TEASED $\times 450$.

- A.—Light stripe.
B.—Dark stripe.
C.—Intermediate segment in light stripe.

FIG. 52.

L.S. CARDIAC MUSCLE (HUMAN), STAINED WITH HÆMATOXYLIN
 $\times 300$.

- a.*—Muscle cells.
b.—Nuclei of same, with yellow pigment at poles.
c.—Fibrous tissue between muscle fibres.
d.—Junctions of segments of muscle.
e.—Capillary blood-vessel.

Fig. 50.



Fig. 51.

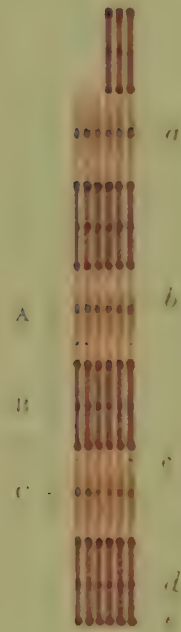
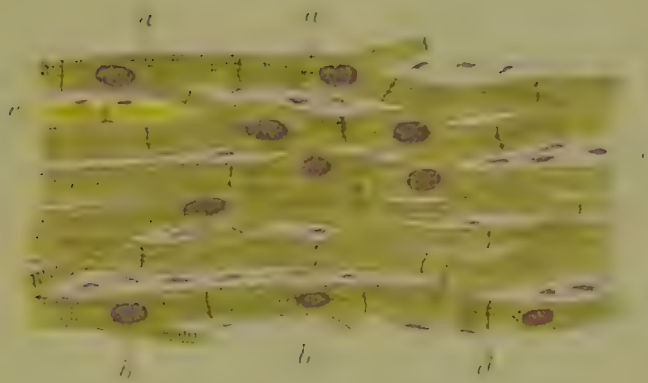


Fig. 52





considerable numbers in the hearts of some ruminants, such as the sheep and ox. They are large, somewhat quadrangular cells. They have one or two nuclei, and apparently consist of ordinary protoplasm, the peripheral part of which shows very distinct striation.

THE STRUCTURE OF NON-STRIPED MUSCLE.

Non-striped muscle is found, generally speaking, where contraction is slow and involuntary. It is thus found throughout the alimentary canal, forming its muscular coats, and giving off slips, which pass from the muscularis mucosæ into the mucosa itself; in the walls of the vascular system—that is, in the arteries and veins; in the ureter, vas deferens, Fallopian tubes and uterus; in the capsule of the spleen and lymphatic glands; in the skin and iris; and in other situations. It has the peculiarity of being under the control of the will, where it forms the muscular wall of the bladder, and the ciliary muscle of the eye.

(1,) The cells are elongated and fusiform in shape, usually unbranched; in length from $\frac{1}{800}$ to $\frac{1}{120}$ of an inch, and in breadth $\frac{1}{8000}$ to $\frac{1}{2500}$. The cells vary in size, however, in different animals and in different situations.

(2,) There is a distinct cell envelope, but no separable sarcolemma. The cell envelope frequently shows transverse creases, which have no relation, however, to the cell contents.

(3,) Each cell possesses one oval or rod shaped nucleus, often with a few granules, extending in a linear manner from its poles. The nucleus is placed in the centre of the cell, which it frequently causes to bulge at the spot.

(4,) The cells lie parallel with each other, and are united together with cement substance, their ends overlapping. The cement substance can be revealed in the ordinary way with nitrate of silver. The cells are arranged in bundles or fasciculi, often of considerable size, surrounded by ordinary connective tissue. The individual fibres or cells are, however, only separated from each other by cement substance, and in this way differ from the two previous forms. The vascular supply is considerably less than that of ordinary skeletal muscle, the small vessels and capillaries forming networks with very much larger meshes.

(5,) The cell substance is faintly fibrillated longitudinally.

Examine the following specimens.

(1.) *Muscle of frog, fresh, teased.*

Tease a small portion of the gastrocnemius or sartorius muscle of the frog in the serum, or in normal saline; cover, and examine first with the low, and then with the high power. Observe the cylindrical shape of the fibres, their breadth, and the appearance of longitudinal and transverse striation, the latter the more distinct. Note that in this fresh specimen of amphibian muscle, simple teasing does not separate the fibres into their constituent fibrillæ; in order to accomplish this the tissue requires previous treatment. Follow one or more of the fibres along its course, and observe their considerable length, only a portion of any one fibre being in the specimen. Look for a spot where the sarcolemma has risen up from the fibre, from imbibition of the fluid outside it, in the form of a bleb, and note its transparency. The bleb is most readily seen when it occurs on the side of a fibre, and appears merely as a line bulging away from it. The nuclei of the cells are scarcely visible at present; but on the addition of a drop of weak acetic acid, by the method of irrigation, they immediately become revealed. Note their considerable numbers, their narrow, elongated shape, and that they run in the long axis of the fibre. A small piece of the same muscle may also be teased in picro-carmin (Fig. 45 A), when the nuclei, which take on the carmine stain deeply, are again shown. The presence of the sarcolemma may easily be demonstrated as follows: Tease a small piece of the sartorius in such a way that the separated fibres are left in somewhat parallel lines on the slide; lay a stiff hair across them, cover, and apply pressure with a needle or some other instrument. The hair is then to be gently withdrawn, without removing the cover-glass. Now examine the specimen for fibres ruptured, as in the figure (B). Note that though the muscular substance proper, *i.e.*, the contents of the sarcolemma, have been divided, and the ends are retracted, the sarcolemma itself (*a*) remains uninjured, and still forms a link between the broken ends. In many cases one part of the fibre becomes turned partly round, twisting the sarcolemma.

(2.) *Muscle of frog, prepared with ammonium carbonate, teased.*
(Fig. 45 C).

Look for the appearance shown in the figure. In many cases the fibres are to be seen merely broken across, but it is usually

quite easy, with a little care, to find places where the transverse cleavage into discs is well seen.

(3.) *Muscle of crab, extended, stained with hæmatoxylin or eosin.*
F. (Figs. 51 and 53 A.)

Tease very thoroughly a small portion of muscle, taken from the extensor aspect of a crab's claw, which has been previously hardened and stained in hæmatoxylin. Mount in Farrant. For the examination of crab's muscle, the student should use as high a power as possible. If only the usual power is available, the tube of the microscope should be drawn out to its fullest extent. Examine the specimen for fibrils, showing the appearance of muscle extended to its full physiological extent. Observe, first of all, the long dark, and light segments. In the latter, look for Dobie's globule, with the "nebenscheibe" of Merkel on each side of it, forming the "intermediate segment"; and between this and the long sarcous element, a clear space with Flögel's granule in it. When the fibrils are seen in lateral apposition, both Dobie's globules and Flögel's granules form lines traversing the light stripe, of which Dobie's is very much the more distinct. Note the most distinguishing characteristic of extended muscle: the light stripe is broad; or, when a single fibril is examined, the light segment is long; and on either side of the central line or globule, as the case may be, there is to be seen another, due to Flögel's granules, or granule. The student should endeavour to recognise all the characters of extended fibrils as described on a previous page, and which need not be recapitulated here. If the fibrils are to be seen broken across in any parts of the specimen, note particularly where the transverse cleavage has taken place. It will always be found to be through the clear segment, between the "nebenscheibe" and the long sarcous element, so that the extremities of the long sarcous elements may form the broken ends of the fibre, or if cleavage has taken place on the other side of Dobie's line, the ends of the intermediate segment may limit it. In such a case, the transverse cleavage often serves as a very good demonstration of the presence of the intermediate segment. The same specimen will probably also show many instances of relaxed unextended fibres, in which the light stripe may be narrower than the dark. In this case Flögel's granules will not be visible, and only Dobie's line, crossing the centre of the light stripe, will be seen.

(4.) *Muscle of crab, contracted, stained with hæmatoxylin or eosin. F. (Fig. 53 F.)*

Tease thoroughly a portion of muscle from the flexor aspect of a crab's claw, previously treated in the same manner as the last. Mount in Farrant.

Examine with the high power. Note the typical appearance of contracted muscle; alternate stripes, light and dark, much narrower than in the previous specimen, the light stripe crossed by a faint line (Hensen's), which must not be confused with Dobie's line in the extended fibre. The light stripe here corresponds with the shafts of the long sarcous elements of the dark stripe in extended muscle, Henson's line appearing in their middle as this becomes light. Dobie's line has now become fused with the heads of the sarcous elements to form the dim stripe of the contracted fibre. Observe the altered contour of the side of a fibre—the alternate convexity and concavity—the former opposite the dark, and the latter the light stripe. In a single fibril or in a small bundle, this results in a very characteristic “beaded” appearance.

(5.) *V.S. Cat's tongue, stained with picro-carmin, mounted in Farrant. (Fig. 101.)*

Mammalian striated muscle may be studied in a section of any of the skeletal muscles. The tongue of a cat or dog is convenient for showing fibres cut both longitudinally and transversely. In a vertical transverse section of cat's tongue, stained with picro-carmin and mounted in Farrant's solution, observe with the low power the way in which the different muscular fibres are cut. Those which run vertically from below upwards, and those which run transversely from the mesial septum outwards, are cut longitudinally, while those running from the base to the tip of the tongue are cut transversely. Put on the high power, and notice the breadth of the longitudinally cut fibres as compared with those of the frog; they are much narrower.

(6.) *T.S. Human muscle, stained with hæmatoxylin. F. or B. (Fig. 49.)*

In such a specimen, observe that the muscular fibres are collected into fasciculi or bundles (cut transversely), and that many of these go to form the whole muscle. The latter is invested with a general fibrous sheath, the epimysium, which sends in septa between the fasciculi—the perimysium, and from the latter delicate connective tissue extends between the fibres themselves—

the endomysium. It is the epimysium which is removed in "cleaning" a muscle in the dissecting room. With the low power, observe first the general arrangement of parts; the epimysium, perimysium, and transversely cut fasciculi of muscular fibres, then put on the high power. Find the epimysium; it is composed mainly of white fibrous tissue with a few elastic fibres. It contains blood-vessels and nerves. Follow one of the septa from it, inwards, and note how it joins with other septa to surround the fasciculi of muscle fibres. Note the outlines of the fasciculi, which are angular, not round, as in the case of nerve bundles. Between the muscular fibres observe the nuclei of the delicate endomysium. The fibrillar part of the endomysium is difficult to make out, but the nuclei of the connective tissue corpuscles between the muscular fibres are obvious. Distinguish them carefully from the nuclei of the muscular fibres themselves; look for a transverse section of a muscular fibre showing a nucleus; observe the angular outline of the transverse section of the muscle fibre; the nucleus will be seen usually to occupy one corner immediately beneath the sarcolemma, in close attachment to the muscle cell. When a nucleus is isolated, and unconnected with any such cell or fibre, it belongs to one of the connective tissue cells of the endomysium.

(7.) *V.S. Injected tongue of cat. B. (Fig. 47.)*

Study vascularisation in the injected muscle of any small mammal, such as a cat. Notice that the arteries break up, as represented in the figure, into an ultimate set of capillaries, which run longitudinally between the muscular fibres themselves, and communicate with each other by transverse branches; also how very perfect the vascularisation is, every muscular fibre being thus brought into intimate relation with the blood stream.

(8.) *Wing muscle of house-fly teased, stained with picro-carmin. F. (showing termination of muscle fibres in tendon). (Fig. 46.)*

There is no gradual transition between tendon and muscle. The tendon fibres are directly attached to the sarcolemma of the conical ends of the muscular fibres. The connective tissue between the muscular and tendinous fasciculi are continuous with each other. Except in specially prepared specimens, however, the appearance is as if the muscle fibre and the tendon bundle passed insensibly into each other.

(9.) *V.S. Left ventricle of heart of man, stained with hæmatoxylin. F. (Figs. 48 and 52.)*

FIG. 53.

MUSCLE OF CRAB, STAINED WITH EOSIN, TEASED $\times 1000$. (SEMI-DIAGRAMMATIC.)

A.—Fully extended fibre.

a.—Dark stripe.

b.—Light „

c.—Intermediate segment.

d.—Flögel's granules.

e.—Dobie's globules.

B.—Relaxed fibre.

c.—Intermediate segment.

C, D, E, F.—Stages of contraction of fibre.

a.—Long sarcous elements.

e.—Dobie's globules.

f.—Hensen's line.

FIG. 54.

A.—NERVE OF FROG, STAINED WITH OSMIC ACID AND PICRO-CARMINE, TEASED $\times 250$.

a.—Medullated fibres. *b.*—Non-medullated fibres. *c.*—Segments of Schmidt. *d.*—Nuclei. *e.*—Node of Ranvier.

B.—L.S. MAMMALIAN NERVE FIBRE AT NODE OF RANVIER, STAINED WITH CARMINE $\times 350$.

f.—Grey sheath. *g.*—Node showing transverse line. *h.*—Axis cylinder.

C.—SEMI-DIAGRAMMATIC REPRESENTATION OF MEDULLATED NERVE FIBRE, STAINED WITH OSMIC ACID AND CARMINE $\times 400$.

i.—Axis cylinder. *k.*—Node of Ranvier. *l.*—Medullary sheath. *m.*—Nucleus. *n.*—Grey sheath.

D.—T.S. NERVE FIBRES, STAINED WITH OSMIC ACID $\times 300$.

E.—RANVIER'S NODE, STAINED WITH AgNO_3 $\times 300$.

F.—PORTION OF NERVE OF FROG, STAINED WITH NITRATE OF SILVER, SHOWING RANVIER'S CROSSES AND OUTLINES OF EPITHELIAL CELLS $\times 50$.

t.—Ranvier's cross. *s.*—Epithelial outline.

Fig. 53.

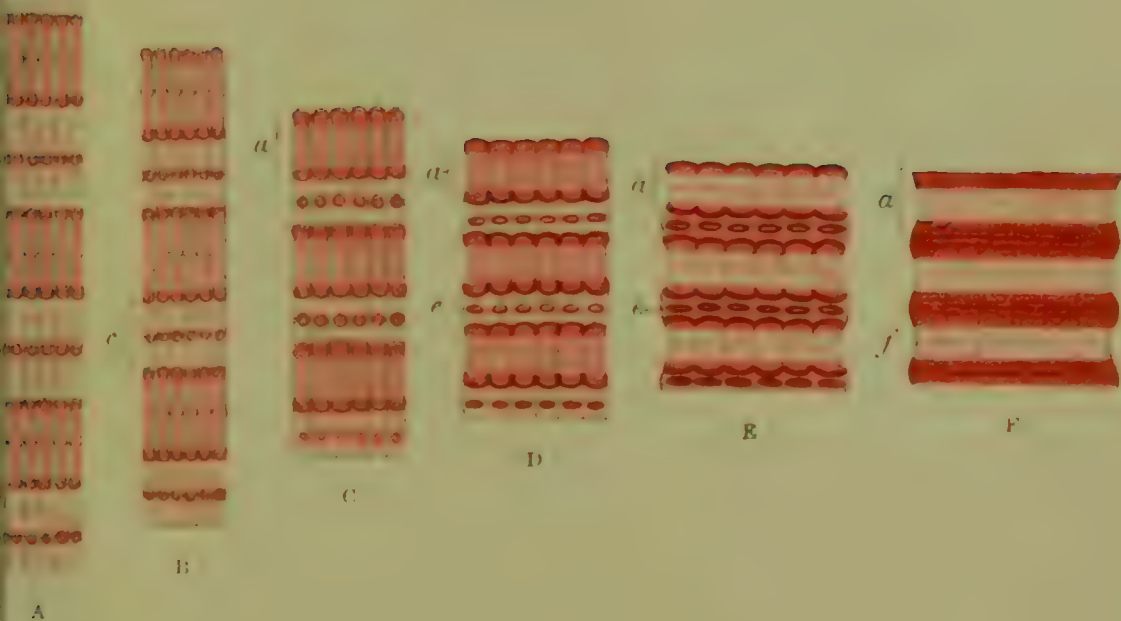


Fig. 54.



Study the structure of cardiac muscle in a section, taken through the left ventricular wall of the heart of sheep or man, from peri- to endocardium. In many parts the fibres will be cut longitudinally, and in many also transversely, especially towards the endocardium, where the knife has passed transversely through some of the columnæ carneæ. Find a part where the fibres are cut longitudinally, and examine it with the high power.

Observe the peculiar network of cardiac fibres. Note that the branch given off from a cell is usually narrower than the body of the cell from which it springs. Notice the oval nucleus, stained blue in the middle of the cells, which, by their union, constitute fibres. Look for the lines indicating the junction of the segments of muscle by their serrated ends. Observe the striation of the fibres, and the presence of connective tissue between them.

It is of the greatest importance to become able to distinguish readily between the transverse sections of the three varieties of muscle fibre; the teased preparations, or sections cut longitudinally, do not present the same difficulty in the way of discriminate recognition as the transverse ones. Note, first of all, the size and shape of the transverse section of a cardiac muscular fibre; it is not so large as that of the skeletal one; and there is considerable variation in size, some of the sections being through the bodies of the cells, and some through their branches; the shape is rather different; there is not the same tendency to a prismatic angular outline, but rather to a round or oval one. The best idea of the shape, however, will be obtained by a study of the specimen itself. The nucleus, when cut through, is seen to be in the centre of the cell. If the knife passes above or below it, or through one of the branches, then the transverse section appears to have no nucleus. Look for a peculiar appearance, which may be seen at the periphery of a section of a muscle fibre, and which is shown in the drawing of fibres cut transversely. There is an appearance as if cleavage into longitudinal fibrils had commenced at the surface of the fibre, had penetrated a short distance, and had then stopped. This is probably the explanation of longitudinal striation, and of the fact that the fibre will not separate into fibrillæ by teasing.

(10.) *V.S. Ventricle of heart of sheep. Picro-carmin. F.*

Beneath the endocardium, look for the groups of large somewhat quadrangular cells — Purkinje's — peculiar to this situation. They stain to a considerable extent with the picric

acid, and are readily recognisable. Under the high power, note the presence, in many, of two nuclei, and identify the striation at the periphery of the cells.

(11,) *Muscular wall of small intestine of cat, teased, stained with hæmatoxylin. F or B. (Fig. 43.)*

Observe under the high power, the long, tapering shape of the cells, the nucleus placed in the centre of each, and the outline indicating the cell envelope. For the cell envelope use as high a power as possible, and endeavour to observe the transverse markings or "crinklins" in it, said to be due to the contractions of the longitudinal fibrillæ.

(12,) *T.S. Small or large intestine of cat, stained with hæmatoxylin or picro-carmin. F or B. For L.S. and T.S., non-striated muscle. (Figs. 118 and 43 b.)*

The circular coat has its fibres cut longitudinally. It is instructive as an instance of L.S. non-striated muscle, but the outlines of individual cells cannot be made out. Note the oval or rod-shaped nuclei.

Examine the same specimen for transverse section of non-striated muscle; the outer longitudinal muscular coat is cut transversely. Observe first, under the low power, that it is cut up into more or less square-shaped blocks, and separated from the internal circular coat by a narrow band of connective tissue, in which lies a plexus of nerves. Put on the high power over a part where the transverse sections of the fibres seem distinct from each other. Notice the general appearance, as of a mosaic. The sections of fibres are quadrangular, prismatic, often diamond shaped; they vary in size according to whether they are cut through the nucleus or a narrower part; in the latter case, of course, there is no nucleus to be seen. There is no connective tissue between the sections of the fibres, only a clear line representing the cement substance. Contrast this section with the corresponding ones of cardiac and skeletal muscle. Observe the small size of the sections of non-striated muscle fibres and the absence of connective tissue between them.

(13,) *Small intestine (muscular wall of) of rabbit, stained with silver nitrate. B. (Fig. 10.)*

The mucosa must be carefully scraped away. It is easily recognised by its velvety appearance. Clear in clove oil in a watch glass; it is safer also to dehydrate in the same way; mount in balsam. First examine with the low, and then with

the high power. Observe the blood-vessels in the muscular wall ; many will have the outlines of their epithelial cells, and the cement between the fibres of their muscular coat, stained with nitrate of silver. Observe, especially, the outlines of the fibres of the muscular wall of the intestine. On focussing downwards a set of parallel lines, representing the cement between the muscular fibres of the uppermost coat (circular) will come into view first, and then, lines crossing these at right angles, belonging to the lower (longitudinal) coat, will be seen.

(14.) *Bladder of salamander, stained with hæmatoxylin and eosin. B. (Fig. 44.)*

Note the muscular fibres, the contour of which is distinctly bulged at the position of the nucleus ; some of the fibres are tri-radiate (*b*). Note the capillaries containing altered blood corpuscles ; and the connective tissue corpuscles of the bladder wall (*d*).

NERVE TISSUE.

There are two main divisions of the nervous system, the cerebro-spinal and the sympathetic. The cerebro-spinal includes the brain, spinal cord, and the nerve trunks passing from them ; the sympathetic the chains of ganglia on either side of the vertebral column, and the visceral plexuses in connection with them. The two systems are in communication with each other, and to some extent commingled.

Nerve tissue is composed of nerve fibres, or of nerve fibres and nerve cells, supported by connective tissue, which varies in character in different situations.

Nerve fibres.—Nerve fibres form the bulk of the nerve trunks and plexuses, and are associated with nerve cells, in the brain, spinal cord, and ganglia.

They are of two kinds, medullated and non-medullated.

Medullated nerve fibres (*Figs. 54 and 56*).—These are found in the white matter of the brain and spinal cord, and form the greater part of the cerebro-spinal nerves. A medullated fibre consists of an axis cylinder, surrounded by a medullary sheath, from which it derives its name. A thin additional outer, or grey sheath, is also present, except in the case of the fibres in the brain and spinal cord.

The axis cylinder is probably of the nature of white fibrous tissue. It is fibrillated longitudinally, the fibrillæ

FIG. 55.

NERVE OF FROG, TEASED, STAINED WITH NITRATE OF SILVER $\times 200$.

a.—Nerve fibres.

b.—Ranvier's crosses.

FIG. 56.

T.S. WHITE MATTER OF SPINAL CORD (HUMAN), STAINED WITH
PICO-CARMINE $\times 350$.

a.—Pia mater.

b.—Septum from pia.

c.—Neuroglia cell.

d.—T.S. Nerve fibre.

On the left side of the septum the supporting tissue is shown
without the nerve fibres.

Fig. 55.

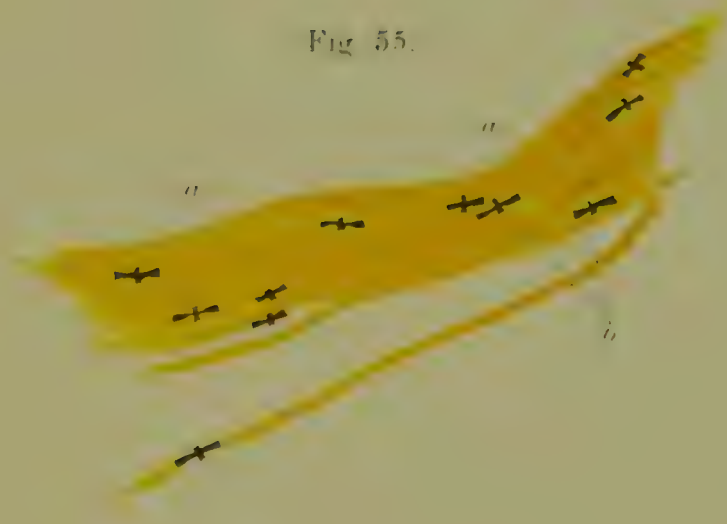
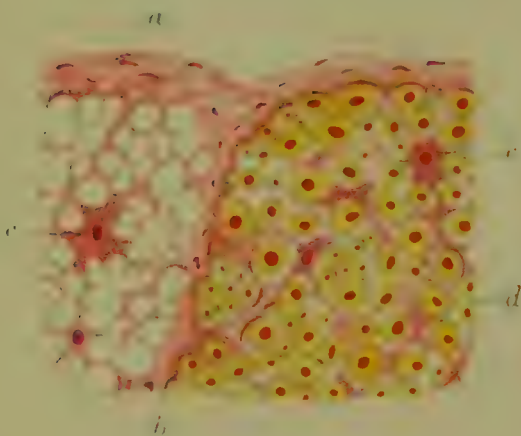


Fig. 56.



being united together with cement substance. It is continuous throughout the length of the fibre, extending from the nerve cell to the periphery. It is the functionally active portion of the fibre.

The medullary sheath surrounding the axis cylinder is interrupted in its course at regularly recurring intervals, and at these points the outer or grey sheath dips inwards towards the axis cylinder, causing a constriction, or *node of Ranvier*. The medullary portion of the internode is again split up, into a series of imbricating segments, by the *incisures of Schmidt* or *Lantermann*. The ends of these segments are either conical or funnel shaped, according as they are overlapped by, or overlap those adjacent to them. In its more minute structure, a segment consists of an outer and inner layer of neuro-keratin, with an intervening network of the same material, the meshes of which contain a fatty material termed myelin. It consists of cerebrin, lecithin, and some other fats in minute quantity. It readily exudes from the cut or broken ends of fibres, and forms highly refractile homogeneous drops, which assume various shapes. By the way in which it refracts light, it gives to the fibre its appearance of a double contour. It is stained black with osmic acid.

Between the medullary sheath and the axis cylinder is a thin layer of cement.

The grey sheath, sheath of Schwann, or neurilemma, is a thin homogeneous, transparent membrane, lying immediately outside the white, or medullary sheath, except where it approaches the axis cylinder, at the nodes of Ranvier.

In each internode, a nucleus is placed immediately beneath the grey sheath, between it and the white, midway between the nodes. It is usually surrounded by a small amount of undifferentiated protoplasm. The part of a fibre between any two nodes corresponds with one of the cells from which it has been developed, the cells being placed end to end, and their junctions forming the nodes of Ranvier. The fibres do not divide, except when approaching their peripheral termination. When division occurs it takes place at a node of Ranvier.

The fibres vary very much in size. Three varieties may be distinguished. In an ordinary cerebro-spinal nerve, most of the fibres are from 8μ . to 16μ . in diameter; those passing from the roots of the spinal nerve to the sympathetic system are

about 2μ . in diameter ; while those of the anterior roots of the spinal nerves are intermediate.

Arrangement of fibres in a cerebro-spinal nerve (Fig. 57).

The nerve fibres are collected into cylindrical fasciculi, or funiculi, of varying size, and a small nerve may be represented by only one of these. A medium sized one, such as the ulnar or median, is composed of several fasciculi, bound together by connective tissue. The latter is variously designated, according to its position, epi-, peri-, and endoneurium. The *epineurium* forms a sheath for the whole nerve, and sends in septa between the fasciculi of fibres. It contains blood-vessels, nerves, and lymphatics, and frequently a number of fat cells. The *perineurium* immediately surrounds each fasciculus, and is represented by several lamellæ of white fibrous tissue, parallel to each other, and separated by a lymph space, lined with simple squamous epithelium. The *endoneurium* consists of very delicate connective tissue, extending from the perineurium, between the nerve fibres themselves. The nerve roots springing from the spinal cord, and the optic nerve, differ from ordinary nerve trunks in the arrangement of the connective tissue. They are invested with an epineurium, or sheath of fibrous tissue, which sends in septa, dividing the nerve into fasciculi. The latter, however, are not cylindrical, but irregularly polygonal in transverse section, and there is an entire absence of a lamellated perineurium.

Non-medullated nerve fibres.—The sympathetic nerves, are mainly composed of non-medullated fibres, with an admixture of small medullated ones. Non-medullated fibres are also found, sparsely intermingled with the medullated, in the cerebro-spinal nerves.

A non-medullated nerve fibre consists of a fibrillated axis cylinder, with nuclei placed here and there along its course, the whole invested by a neurilemma or grey sheath. It differs also from the medullated, in branching and anastomosing with other fibres. The medullated nerve fibres branch only previous to their termination.

A strand of non-medullated fibres is surrounded with a connective tissue sheath—the epineurium ; delicate connective tissue—the endoneurium—is found in small quantity between the fibres themselves.

Nerve cells.—Nerve cells are found in the grey matter of the central nervous system, and in ganglia in different parts of the

body. In structure they vary very much, according to the position in which they occur. They are found in the following forms :—

- 1.—*Uni- and bi-polar nerve cells, found in the ganglia on the posterior roots of the spinal nerves.*
- 2.—*Multipolar nerve cells of the sympathetic chain of ganglia.*
- 3.—*Multipolar nerve cells of the grey matter of the spinal cord.*
- 4.—*Pyramidal multipolar nerve cells, of the cortex of the cerebrum.*
- 5.—*Antler multipolar nerve cells, of the cortex of the cerebellum.*

(1.) *Nerve cells of the spinal ganglia. (Fig. 59 a, b.)*

The nerve cells of the spinal ganglia differ in shape in different animals. The typical bi-polar cell is well seen in the skate. The two processes spring from opposite sides of the cell, which is nucleated and nucleolated, spindle-shaped at the poles, and invested with a nucleated capsule, continuous with the grey sheath, or neurilemma of the nerve fibre. The nuclei of the capsule are the representatives of the nuclei upon the nerve fibre. Both processes are "axis cylinder" processes, *i.e.*, they acquire a medullary sheath, and thus become medullated nerves. A more unusual form of bi-polar nerve cell is that described by Beale, found in the spinal ganglia of the frog. Here the cell is pyriform in shape, and the second fibre arises from the same end as the first, which may be considered the stalk of the pear. It coils spirally round it for a variable distance, and finally leaves it, to proceed in an opposite direction. The cell is nucleated as before, and invested with a nucleated sheath, continuous with the grey sheath of the two fibres in connection with it. Both of these are axis cylinder processes, and acquire medullary sheaths.

The so-called unipolar nerve cells are found in the spinal ganglia of mammals. They are only unipolar, however, in a half sense, *i.e.*, the fusion between the two fibres takes place a short distance before they reach the cell, which they enter as one nerve. The fusion may take place at the last node of Ranvier, or at the last but one, two, or three. The cells are round in shape, nucleated, and invested with a nucleated capsule. Both processes become the axis cylinders of medullated nerves. The cells are probably virtually bi-polar, with the afferent and efferent fibres fused together for a short distance before they join the cell.

(2.) *Nerve cells of the sympathetic ganglia.*

These are cells with a variable number of processes, each of which appears to be continued as a non-medullated fibre, or one may acquire a medullary sheath, and become the axis cylinder of a small medullated nerve fibre. The cells are nucleated, and possess a nucleated capsule, continuous with the grey sheath of the nerves passing from them. In consequence of the number of the processes, the cells are more irregular in shape than in the case of uni- and bi-polar nerve cells.

(3.) *Nerve cells of the spinal cord.* (Fig. 59 c.)

Those found in the anterior cornua of grey matter are the largest, and the most typical of a multipolar nerve cell. Those in the posterior cornua are much smaller, and, in the case of Clarke's column, more fusiform in shape. A nerve cell from the anterior horn is large, irregular in shape, and multipolar. It possesses a nucleus and nucleolus. Of its many processes, one only becomes the axis cylinder of a medullated fibre. The remainder, the peripheral or grey processes, break up into a fine network of fibrils, which anastomose with each other and with the fibrils from adjacent cells. None of the cells are encapsuled, and none of the processes possess a grey sheath. The fibrillation of the axis cylinder and grey processes is particularly well seen in cover-glass specimens, stained with methyl-blue; and in the cell itself the fibrils continued from the processes can be seen crossing each other at various angles, to form a very beautiful network, and very frequently be traced from one process into neighbouring ones. No special connection between the network and the nucleus of the cell has been established.

(4.) *Nerve cells of the cortex of the cerebrum.* (Fig. 59 e.)

These cells have a more constant shape than those of the grey matter of the spinal cord. They are, roughly speaking, of the form of a pyramid with a narrow base, to the centre of which the single axis cylinder process is attached. The rest of the processes, springing from the sides of the pyramid, from its apex, and from the periphery of its base, are peripheral grey processes, which branch and anastomose, in a manner similar to those of the nerve cells of the spinal cord. These pyramidal cells vary in size, according to the layer of the cortex to which they belong. Like the multipolar nerve cells of the spinal cord, they are nucleated and nucleolated, and possess no sheath or capsule.

(5.) *Nerve cells of the cortex of the cerebellum.* (Fig. 59 d.)

The "antler cells" of the cortex of the cerebellum are so called, from the appearance and arrangement of their peripheral processes. These spring more particularly from the top of the cell, or that directed to the surface of the convolution, and from their disposition and mode of branching have been likened to the antlers of a stag. They arise as one or more broad trunks, directed towards the surface, dividing as they go into numerous branches, many of which run a short distance transversely, and then continue their vertical course. The axis cylinder process enters the usually pointed base of the cell obliquely from below. The cells are nucleated and nucleolated, but without a capsule. They do not vary much in size.

Supporting tissue of the central nervous system.—Neuroglia is found, both in the grey and white matter of the central nervous system, serving as a support for the proper nervous elements. In many ways it is comparable with the endoneurium of a cerebro-spinal nerve. It is not, however, strictly speaking, of the nature of connective tissue, as it is developed, not from the mesoblast, but the epiblast. It consists of a number of large nucleated cells; with greatly ramified processes, conforming in their disposition with the nerve elements, cells, or fibres, or both, which they serve to support, and forming so delicate a network as to present almost a molecular or granular appearance.

The pia mater also sends inwards prolongations of its tissue, which lend support to the nerve elements among which they pass.

Examine the following specimens:—

(1.) *Fresh nerve of frog, teased in normal saline.*

Take a small portion of the sciatic nerve of a frog, tease in normal saline, cover, and examine under the high power.

Observe the double contour of the nerve fibres, and note that when treated thus the margin does not remain straight, but becomes more or less curved, and irregular. The double contour represents the medullary sheath. The axis cylinder is scarcely visible in this preparation. Select one of the fibres, and follow it along until an interruption in the medullary sheath, a node of Ranvier, is found. It may be necessary to examine several fibres in succession for this purpose. It is only at this point, as a rule, that the outer or grey sheath can be seen, as it is too closely applied to the medullary sheath to be observable in the internode. The incisures and segments of Schmidt are better seen in the next preparation.

FIG. 57.

T.S. CEREBRO-SPINAL NERVE (HUMAN), STAINED WITH HÆMATOXYLIN
× 50.

- a.*—Epineurium.
- b.*—Perineurium.
- c.*—Endoneurium.

FIG. 58.

L.S. PORTION OF SCIATIC NERVE (HUMAN), STAINED WITH
HÆMATOXYLIN × 50.

- a.*—Epineurium.
- b.*—Perineurium.
- c.*—Small artery.
- d.*—Bundle of nerve fibres--a "funiculus."

Fig. 57.

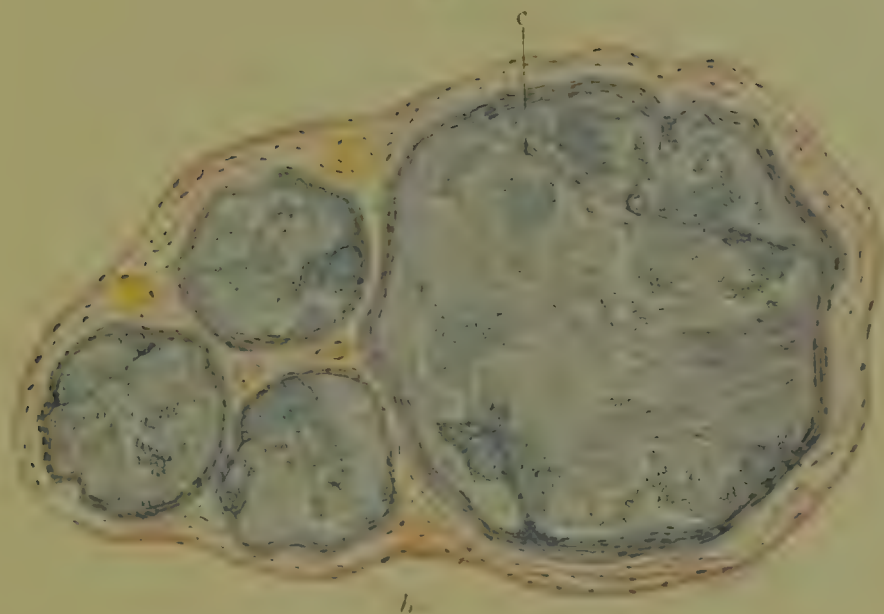
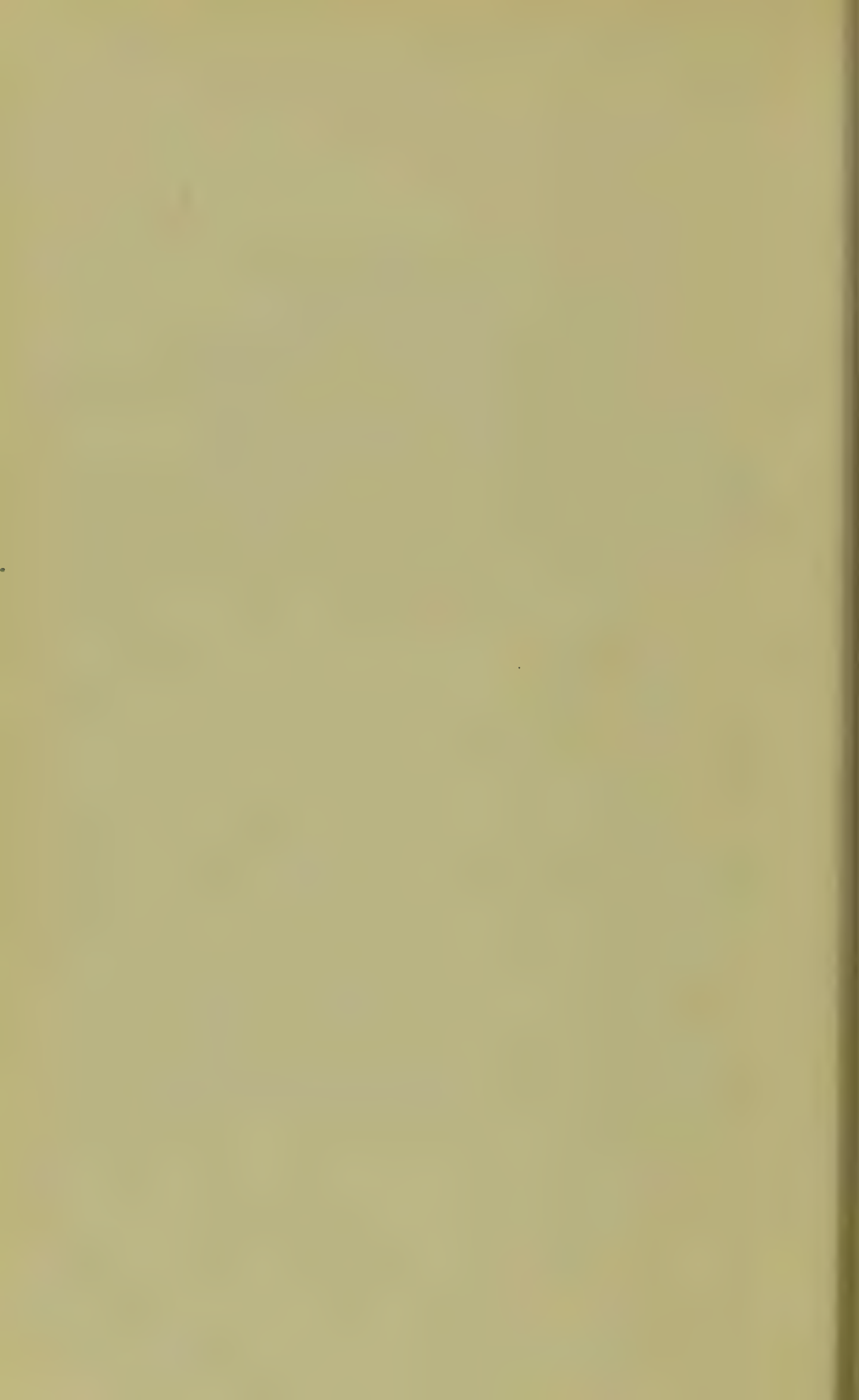


Fig. 58.



(2,) *Nerve of frog, stained with picro-carmin and osmic acid, teased. F. (Fig. 54 A, c.)*

Stain a portion of the sciatic nerve of a frog for twenty-four hours in a .5 per cent. solution of osmic acid, and then for twenty-four hours in picro-carmin. Tease, and mount in Farrant's solution.

Under the high power, note the medullary sheath stained brown or black with the osmic acid, forming a dark broad border to the fibres, the central part of which is more lightly stained. Here and there, the axis cylinder may be traced out, stained slightly with the carmin.

Find one of the nodes of Ranvier, and observe the cone-shaped ends of the segments of Schmidt at this point. Observe the division of the internode portion of the fibre into segments; the obliquity of the dividing *incisures*, and that they are narrower at their inner than their outer ends. About the middle of an internode, look for a red stained nucleus, between the grey and white sheath, *i.e.*, applied to the outside of the dark border.

Look for non-medullated nerve fibres, much narrower than the foregoing, unstained with the osmic acid, or only slightly so, and marked by swellings of an oval shape (nuclei) in their course.

(3,) *Nerve of frog, stained with silver nitrate, teased. (Figs. 54 E, F, and 55.)*

Tease a portion of the fresh sciatic nerve of a frog in silver nitrate ($\frac{1}{4}$ per cent.), wash rapidly with a few drops of distilled water, mount in Farrant's solution, and expose to light.

Or, stain a portion of fresh unteased nerve in silver nitrate ($\frac{1}{4}$ per cent.) for three hours, slightly fray out one end on a dry slide, dehydrate, mount in balsam, and expose to light.

The silver nitrate is reduced by the cement substance, to which it has ready access in the position of Ranvier's nodes, between the axis cylinder and the grey sheath. It also penetrates a little way on either side of the node, along the axis cylinder, between it and the medullary sheath, and is reduced by the cement substance here. In this way we have the appearance of a cross—Ranvier's cross—produced; the short transverse bar being due to the reduction of the silver salt by the cement substance, at the node itself, and the longer vertical one by that between the axis cylinder and the medullary sheath. Examine the unteased end of the second specimen for the outlines of the epithelial cells covering it. They are brought into view

by the reduction of the silver nitrate, by the cement substance uniting their edges.

(4.) *T. S. Cerebro-spinal nerve of man, stained with hæmatoxylin. B. (Fig. 57.)*

Under the low power, examine the specimen first for the general arrangement of parts. Note the fibrous investment or epineurium (*a*) surrounding the whole nerve, and continuous with the fibrous tissue between the nerve funiculi. In this tissue, observe blood-vessels and lymphatics, and in the case of many nerves, large numbers of fat cells. Around each nerve bundle, note a lamellated condensation of fibrous tissue—the perineurium (*b*). The nerve bundles themselves are round in section, and vary considerably in size. Note that their transverse sections are split up into a certain number of sub-divisions, by processes (*c*) of connective tissue—the endoneurium—passing in from the perineurial sheath. From these main processes finer filaments extend between the individual nerve fibres.

Under the high power, identify the parts already referred to, and study particularly the perineurium and the fasciculi of nerve fibres. If possible, find a part where the lamellæ of the perineurium of a bundle have been artificially separated from each other. Note their thinness and delicacy, and the nuclei, stained blue, of the flattened cells covering their surfaces. Study the transverse sections of the nerve fibres in a fasciculus. Note the variations in size; the axis cylinder in the centre of each, stained blue; a clear ring outside it representing the unstained medullary sheath and neurilemma; between the fibres, nuclei of the cells of the delicate endoneurium.

(5.) *T. S. Cerebro-spinal nerve, stained with osmic acid. B. (Fig. 54 D.)*

In such a section, observe under the high power the transverse sections of the nerve fibres with the medullary sheath stained black with the osmic acid. In this way we have the appearance of a number of black rings with an unstained centre, the converse, in fact, of the preceding staining. Note the different sizes of the nerve fibres, which are here particularly obvious.

In this and the former specimen, it will be seen that in a funiculus or cylinder of nerve fibres, all are not cut transversely. This is shown in *Fig. 57*. In some parts of a funiculus the fibres will be cut transversely, while in others they are

cut obliquely or longitudinally. In some cases, all the fibres of a funiculus are cut in the same direction. This is particularly the case when the bundles are small. It is usual to find in an ordinary cerebro-spinal nerve, such as the sciatic, that most of the bundles have their constituent fibres cut differently in different areas. The reason of this will be more apparent in the succeeding preparation.

(6,) *L. S. Cerebro-spinal nerve, human, stained with hæmatoxylin. B. (Fig. 58.)*

It is sufficient to mount one funiculus with its perineurium. Under the low power, note the perineurial connective tissue (*b*) on either side of the bundle of nerve fibres. Note the wavy course of the latter as represented in the figure. They are not straight, unless stretched artificially, but pursue a somewhat zig-zag course, and it is to this that a nerve owes its "watered silk" appearance when looked at with the naked eye. It is this change in the direction of the fibres that causes the appearance of alternate light and dark stripes seen through the epineurium. It is to this, also, that the variation in the way in which the individual fibres are cut in transverse sections is due. The rationale of this will be at once apparent. When the fibres in one part of a nerve (or funiculus of a nerve) are running vertically, in another they may be running so obliquely as to be almost transverse. A transverse section of the whole nerve will cut the first transversely, and the latter obliquely or longitudinally.

With the high power, examine the individual nerve fibres, and more especially Ranvier's nodes. (*Fig. 54 B.*) They are very readily seen as constrictions in the course of the fibres; and this specimen is particularly valuable in that the partition line indicating the junction of two cells at a node of Ranvier is very clearly seen. It occupies the position of the transverse portion of Ranvier's cross, but is a sharply-defined line, and not a broad bar. Look for the axis cylinder of a fibre stained with the hæmatoxylin.

(7,) *Splenic nerve of sheep or ox, teased.*

Tease a small portion of the nerve hardened in Müller's fluid in picro-carmin. Mount in Farrant.

Under the high power, note the small number of medullated fibres. Most of the fibres are non-medullated. They are finely striated longitudinally, and show here and there upon their

course, oval nuclei. Here and there, note that the fibres may be seen to branch. Look for connective tissue cells and fibrils between the nerve fibres.

(8,) *T. S. Splenic nerve of sheep or ox, stained with hæmatoxylin. B.*

Under the high power, observe the transverse sections of the axis cylinders of the fibres, stained with hæmatoxylin, as in the case of the medullated nerve; and note also that they are not surrounded by the clear unstained circular area which is indicative of the presence of a medullary sheath. Note, between the transverse sections of axis cylinders, connective tissue nuclei, and the epineurium surrounding the whole nerve.

(9,) *Muscular coat of small intestine of rabbit, for Auerbach's plexus, stained with gold chloride. (Fig. 120 B.)*

Auerbach's plexus lies between the two muscular coats, and usually adheres to the outer, when the two are separated. Note first with the low, and then with the high power, the large meshes of the network composed of strands of non-medullated fibres. Observe that from these strands or trabeculæ numerous delicate branching anastomosing fibrils (*b*) are given off, in a direction parallel to each other, but usually at right angles to the strands from which they spring. Embedded amongst the fibres, at the junctions of the trabeculæ, note the presence of groups of nerve cells. The nerve fibres of the trabeculæ, and the delicate branches given off from their sides, are stained purple with the gold chloride, while the nuclei of the nerve cells are unstained, looking like little groups of oval, white seeds. Compare this staining with that of the similarly treated specimen of teased tendon, in which the nuclei of the cells were also unstained by the reagent.

(10,) *Spinal ganglion of skate, teased, stained with methyl-blue. (Fig. 59 b.)*

Tease a small portion of a spinal ganglion of a skate, previously hardened; stain with methyl-blue, and mount in Farrant's solution. Under the low power, observe the tangled mass of medullated nerve fibres, with the globular-looking nerve cells occurring frequently in their course. Select a nerve cell which shows a fibre passing from it at each end, and examine it with the high power. Observe, as represented in the figure, the globular spindle shape of the cell, the large nucleus, the nucleolus, the axis cylinder process from each

pole of the cell, and the delicate, grey or outer sheath, enveloping the whole.

(11,) *T. S. Softened spinal column of kitten, or other small mammal, for mammalian spinal ganglion, stained with picrocarmine. F.*

With the low power, or the naked eye, observe in the centre the transverse section of the spinal cord, and around it the bone of the vertebral column. Make out if there is a posterior nerve root cut, showing the ganglion upon it. The section may have passed either above or below the ganglion, and in that case another specimen will require to be mounted. The ganglion may easily be recognised under the low power as an enlargement, upon the posterior root, in its passage through the spinal column, containing a great number of small, round, nucleated bodies—the ganglion cells. Select a portion of the ganglion, where the cells are well seen, and put on the high power (*Fig. 59 a*). Note the round shape of the cells—their nucleus and nucleolus, and, if possible, a process coming off from one of them (*f*). This is usually difficult to find, the cells appearing to be without processes, owing to the way in which they are cut. If a pear with its stalk be taken to represent the cell and its process, it will be readily understood how great the chances are against the knife passing in the long axis of the stalk or process. Observe that the nerve cells are each surrounded with a nucleated capsule—a capsule nucleated on the inside, separating them from the nerve fibres passing through the ganglion, by which they are surrounded. The nerve fibres are cut some transversely and some longitudinally, according to the part of the specimen examined. Notice their nuclei stained with the carmine. At the periphery of the ganglion, observe its fibrous investment, continuous with the epineurium of the nerve root. Such a ganglion is merely an enlargement of the root at a certain point, due to the occurrence there of numerous ganglion cells. The ganglion cells are, for the most part, at the circumference of such a swelling, and the nerve fibres in the centre.

(12,) *Spinal ganglion of frog, teased, stained with picrocarmine. F.*

Isolate one of the small grey ganglia, along the side of the spinal column of the frog, from beneath its calcareous sac; tease in $\frac{1}{4}$ per cent. osmic acid; stain in picrocarmine; mount in Farrant.

FIG. 59.

NERVE CELLS, VARIOUSLY STAINED, HIGH POWER.

- a.*—Multipolar nerve cells from spinal ganglion, stained with hæmatoxylin.
- b.*—Bi-polar nerve cell from spinal ganglion of skate, stained with methyl-blue.
- c.*—Multipolar nerve cell from anterior horn of spinal cord of ox, stained with picro-carmin.
- d.*—Purkinje's (antler) cell from cerebellum (human), stained with carmine.
- e.*—Large pyramidal nerve cell from cortex of cerebrum (human), stained with blue-black.
- e'*.—Small pyramidal nerve cell from cortex of cerebrum (human), stained with blue-black.
- f.*—Axis cylinder process.

FIG. 60.

T.S. SMALL ARTERY, STAINED WITH PICO-CARMINE $\times 250$.

- a.*—Epithelium lining artery, covering the internal surface of intima.
- b.*—Internal elastic lamina, forming the outermost part of the intima.
- c.*—Middle or muscular coat.
- d.*—Adventitia.

Fig. 59.

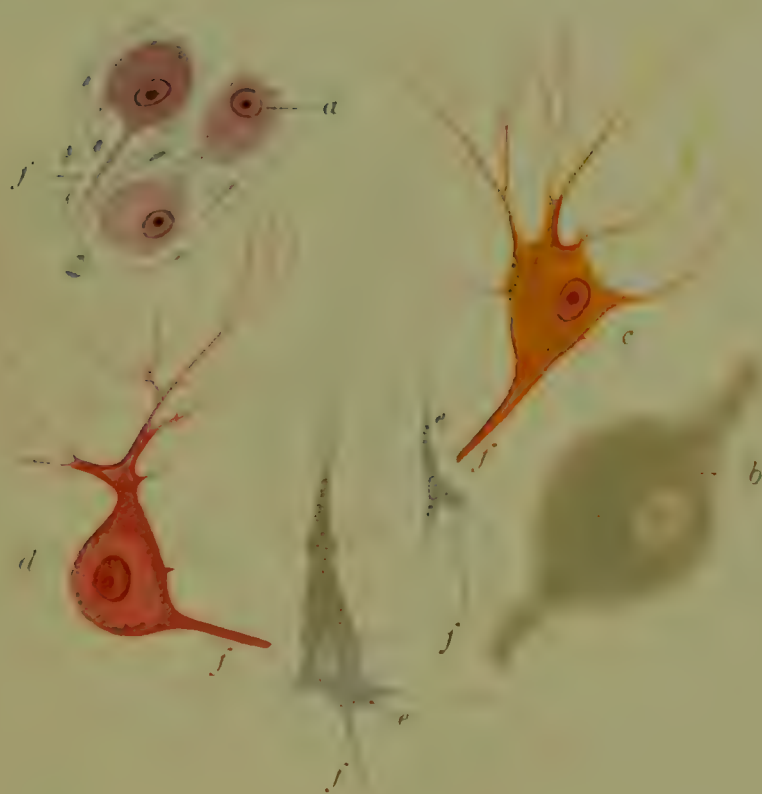
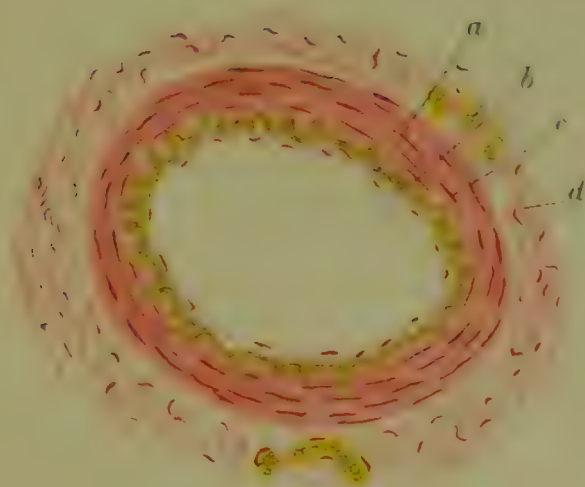


Fig. 60.



Under the high power, look for large round unipolar nerve cells. Each cell is nucleated, nucleolated, encapsuled, and possesses one process, which is usually, however, detached.

(13.) *Section of sympathetic ganglion of man, stained with picro-carmin.* F.

Under the low power, observe somewhat similar features to those found in the section of spinal ganglion of kitten: the numerous nerve cells; the nerve fibres around them cut in various directions; the fibrous tissue investment of the ganglion. Note, in addition, the presence of fat cells belonging to the pre-vertebral fat. With the high power, examine one or more of the nerve cells, and observe that they are inclined to be round in shape, but that their margin is more or less irregular, due to the several processes coming off from it and passing through the capsule. The cells appear somewhat shrunken, with a space between them and their capsules, to which they appear attached by these processes.

This specimen may also be stained in hæmatoxylin, and mounted in balsam. Weigert's method often yields a very beautiful preparation.

(14.) *Anterior horn of grey matter of spinal cord of ox, teased, stained with picro-carmin.* F. (Fig. 59 c.)

Harden a piece of fresh cord in bichromate of potassium, 2 per cent., for a few days; with the point of a scalpel remove a small piece of the anterior cornu; tease in picro-carmin; mount in Farrant.

With the low power, select one of the large multipolar nerve cells, which may be seen deeply stained with the reagent, scattered over the field. Put on the high power, and make out its chief characteristics; its large size; its nucleus and nucleolus; its numerous branching peripheral processes, and that of its single, unbranched axis-cylinder. The latter is narrower than the peripheral processes—that is, at their commencement—and is more deeply stained. It is not, however, always easy to distinguish it from the rest, unless it happens to remain unbroken for a considerable distance from the cell. When broken off short, there is little to distinguish it. Note that the cells have no capsules.

(15.) *Anterior horn of grey matter of spinal cord of ox. Cover-glass preparation, stained with methyl-blue.* B.

In order to study the fibrillation of the processes, and the

fibrillar network in the body of the cell itself, make a cover-glass preparation of the fresh, grey matter of the anterior horn, and stain it with methyl-blue ; mount in balsam ; select a cell, and put on the high power. The fibrils of the processes are stained blue ; trace them into the cell, where they cross others entering from other processes at various angles. Note that they have no obvious relation to the nucleus.

(16,) *Section of cerebrum for pyramidal cells.* (Fig. 174 B.) See "Central Nervous System."

(17,) *Section of cerebellum for antler cells.* (Fig. 174 A.) See "Central Nervous System."

APPENDIX TO CHAPTER VII.
METHODS OF PREPARATION.
MUSCLE.

1. Cleavage of muscle fibres into discs.—Place pieces of frog's muscle in saturated solution of ammonium carbonate for a night ; tease, and mount ; or macerate in dilute hydrochloric acid for some days.

2. Crab's muscle extended or relaxed.—Use the large segment of the small claws. The crab should be obtained alive. Remove one of the small claws, and tie it in the flexed position, *i.e.*, with the second segment bent on the first. Leave for four or five hours, for the muscle to die. Then remove part of the carapace of the first segment, to admit the hardening fluid, and place in Müller's fluid. On the second day transfer to Müller and spirit, and keep for a fortnight, the fluid being changed as often as necessary. See that there is a considerable excess of the fluid ; then transfer to methylated spirit. At the end of a month, transfer to absolute alcohol. Tease small portions of the extensor muscle in eosin, or stain in weak hæmatoxylin for twenty-four hours ; tease, and mount in Farrant. The fibres may also be stained with methyl-blue.

3. Crab's muscle contracted.—Use one of the small claws. Remove the carapace from the anterior aspect of the first segment, so as to expose the extensor muscle. Bind the claw to a piece of wood to retain it in the extended position, *i.e.*, so that the exposed muscle may contract ; place at once in Müller's fluid. The reagent stimulates the living muscle fibres and causes them to contract. Harden it subsequently in the same way as No. 2. Stain with eosin, hæmatoxylin, or methyl-blue.

4. Crab's muscle for fibres in various conditions.—Take one of the small claws of the living crab, fracture the carapace of the first segment so as to admit the fluid and place direct into absolute alcohol. The tissue is ready for teasing in twenty-four to forty-eight hours. Strain as before. Methods 2 and 3, however, secure the best results.

5. Cat's tongue for sections of muscle fibres.—Harden in Müller's fluid, stain in picro-carmin, and mount in Farrant ; or in hæmatoxylin, and mount in balsam.

6. T.S. Human muscle.—Harden a small piece of one of the muscles of the forearm in Müller and spirit ; cut in gum, stain with hæmatoxylin, and mount in balsam. It is very liable, however, when cut in this way, to fall to pieces, and it is, perhaps, better to stain in bulk with borax-carmin, and cut in paraffin.

7. Tongue of cat, injected.—Harden in Müller's fluid, cut in gum, and mount in balsam. Injected sections should be cut rather thick than otherwise, as a certain depth of tissue is not inadvisable; the blood-vessels containing the injection mass are the better shown, but thorough dehydration and clearing are necessary.

8. Ventricle of heart (human).—Harden a piece of the left ventricle, cut from peri- to endocardium, in Müller's fluid, or Müller and spirit; cut in gum, stain in picro-carmine or hæmatoxylin, mount in Farrant. The ventricle of the sheep's heart for Purkinje's cells may be prepared in the same way. Stain in picro-carmine.

9. Isolated cells of non-striped muscle.—Macerate a piece of the muscular coat of the small intestine of a cat in $\frac{1}{2}$ per cent. solution of potassium bichromate, for two or three days; preserve in spirit. Stain for twenty-four to forty-eight hours in weak hæmatoxylin. Tease a shred in Farrant solution; cover.

10. T.S. Small intestine of cat.—Harden the small intestine in Müller's fluid, and then Müller and spirit. Cut in gum, stain in hæmatoxylin, and mount in Farrant or balsam; or stain in bulk in borax-carmine, and cut in paraffin.

11. Small intestine of rabbit injected with nitrate of silver, to show cement lines between non-striped muscle cells. (See page 121.)

NERVE.

1. Cerebro-spinal nerve for T.S. and L.S.—Harden in 2 per cent. ammonium bichromate, or in Müller's fluid; complete in alcohol. Cut in gum, stain with hæmatoxylin, and mount in balsam; or stain in bulk in borax-carmine, and cut in paraffin. If the sections are to be stained in osmic acid, alcohol is better avoided in hardening, as it dissolves out the myelin of the medullated sheath.

2. Splenic nerve of sheep.—Harden a piece of the spleen of a sheep with the splenic nerve in connection with it, in Müller or Müller and spirit.

3. Spinal column of kitten for ganglion on posterior nerve root.—Harden a piece of the spinal column of a half-grown kitten in Müller and spirit; then soften the bony parts in chromic and nitric fluid. Cut in gum; stain in picro-carmine or hæmatoxylin; or stain in bulk in borax-carmine, and cut in paraffin.

4. Sympathetic ganglion of man.—Same as No. 1. Also stain by Weigert's method; cut in gum, and mount in balsam.

5. Teased preparation of cells of anterior horn of spinal cord of ox or sheep.—Harden small pieces of the anterior horn in $\frac{1}{4}$ per cent. osmic acid for a few hours; stain for twenty-four hours in picro-carmine, tease, and mount in Farrant. Or harden in 2 per cent. bichromate of potassium for a few days.

6. Cover-glass preparation of cells of anterior horn.—The cord may be used fresh, or after twenty-four hours in dilute (one-third) alcohol.

CHAPTER VIII.

THE VASCULAR AND LYMPHATIC SYSTEMS.

THE VASCULAR SYSTEM.

THE HEART.

FROM without, inwards the wall of the heart consists of the following layers :—

1.—*The Pericardium* ; 2.—*The Myocardium* ; 3.—*The Endocardium*.

1.—**The Pericardium.**—The heart, like most of the other viscera contained in the cavity of the thorax or abdomen, is invested with a serous sac composed of two layers ; a parietal, applied to the walls of the pleural mediastina, and a visceral layer covering the organ itself.

The two are continuous with each other upon the roots of the various vessels which enter and leave the heart. The visceral pericardium, sometimes termed the ‘epicardium’—which, by its epithelial surface, affords the smooth glistening appearance to the exposed heart, when the chest wall and parietal layer are opened—consists of an external layer of flat, polygonal, squamous cells with an internal fibrous layer beneath it, constituted for the most part of white fibrous tissue, with a considerable amount of the elastic element amongst it in the form of fibres. This layer contains capillaries, lymphatics and nerves, and is continuous with the connective tissue between the muscular fibres of the myocardium beneath it.

2.—**The Myocardium** consists of a network of muscular fibres, the nature of which has already been described under the heading of cardiac muscle (*page 187*). Between the fibres is a certain amount of delicate, connective tissue, continuous on the one hand

with the pericardium, and on the other with the endocardium, and containing blood capillaries, lymphatic spaces and nerves.

3.—The Endocardium resembles the pericardium in structure, but is much thinner. It consists of an inner endothelial lining with a layer of sub-endothelial connective tissue, continuous with that supporting the muscular substance of the myocardium. The endocardium differs from the intima of an artery in containing capillary blood-vessels and lymphatics. The epithelial lining also, like that of the pericardium, consists of polygonal instead of fusiform squames.

The Valves of the Heart.—The auriculo-ventricular valves of the heart and the semi-lunar valves of the aorta and pulmonary artery may be regarded, structurally, as reduplications of the lining membrane, or endocardium. They are composed of fibrous tissue, for the most part of the white variety, with a layer of flattened epithelial cells on each surface. The valves are supplied with capillary blood-vessels like the rest of the endocardium. In the case of the semilunar valves, however, these only extend a little way into the thicker attached portion of the membrane, leaving the rest extra-vascular.

THE BLOOD-VESSELS.

Structure of a Medium-Sized Artery.—(*Figs. 60 and 62*).—One of the smaller arteries, such as the radial, cerebral, or lingual, has the following structure :—

It consists of three coats ; of which the internal, or *intima*, presents a smooth surface to the blood stream, and is also elastic, if the internal elastic lamina is considered part of it, as it usually is ; the middle coat, or *media*, is contractile and elastic ; and the external coat, or *adventitia*, is elastic, and also bears the blood-vessels (*vasa vasorum*) and nerves for the vessel itself.

1.—The Intima consists of these parts : (*a*,) An *endothelial lining* of flattened elongated cells, with a somewhat sinuous outline, cemented one to another by their margins, their long axis parallel with that of the vessel they line ; (*b*,) *Sub-endothelial connective tissue*. Beneath the epithelium is a layer of connective tissue, which varies in nature and extent according to the size of the artery in question. In such as those mentioned above, this consists of a non-vascular, narrow layer of ordinary connective tissue, bounded on the out-

side with a tubular homogeneous elastic membrane—the *internal elastic lamina*. This is frequently of the nature of a fenestrated membrane of felted elastic fibres, but it may be a complete membrane, and show the merest trace of its fibrous origin.

The smaller the artery, the less of the sub-endothelial connective tissue is there, until, ultimately, the epithelium lies directly upon the internal elastic lamina, which, in that case, occupies the position of a basement membrane. In a transverse section of a medium sized artery, such as we see in ordinary specimens of prepared tissues, the internal elastic lamina appears as a highly refractile sinuous band, surrounding the lumen of the vessel, bearing on its surface the rest of the intima, which may be represented by epithelium alone, or by epithelium with a layer of white fibrous tissue beneath it. The appearance of sinuosity, which the internal elastic lamina presents in section, is due to the fluting the tube undergoes, owing to the diminution in the lumen of the vessel, when the pressure of the fluid within it is removed.

2.—The **Media**, or middle coat of the artery, is the broadest of the three, and is composed of circularly arranged, non-striped muscle fibres, amongst the layers of which are found a varying number of elastic plates, and a small amount of delicate white fibrous tissue. The plates vary in proportion to the muscular substance, according to the size of the artery, *i.e.*, to the more special function it is required to subserve. In the case of the aorta, especially, and the larger arteries as well, the function of the vessel is to a much greater extent of an elastic nature than in the case of the smaller vessels, such as the lingual or radial, in which it is more muscular; there may even be a preponderance of elastic and fibrous tissue over the muscular element in the media of the former, whereas in the latter, the muscular element is always in excess. The elastic plates are structurally of the same nature as the internal elastic lamina; that is to say, they are fenestrated membranes or felt-works of elastic fibres. In transverse section, they appear as highly refractile sinuous lines amongst the non-striped muscle fibres—the sinuosity, as in the case of the internal elastic lamina, being due to the longitudinal fluting of the plate.

3.—The **Adventitia**, or external coat, consists of white fibrous tissue, with a network of elastic fibres running for the most part in a longitudinal direction. In this coat the *vasa vasorum*, or

FIG. 61.

S. AORTA OF DOG, STAINED WITH Picro-Carmine $\times 200$.

- a.*—Intima.
- a*¹.—Epithelium.
- b.*—Media.
- b*¹.—Elastic plates.
- c.*—Adventitia.
- c*¹.—Elastic fibres cut transversely.

FIG. 62.

A.—T.S. RADIAL ARTERY (HUMAN), STAINED WITH Picro-Carmine
 $\times 350$.

B.—T.S. VEIN, STAINED WITH Picro-Carmine $\times 350$.

- a.*—Intima.
- b.*—Media.
- c.*—Adventitia.
- d.*—Internal elastic lamina.
- e.*—Elastic plates in media.
- f.*—Nuclei of muscle cells.
- g.*—Vasa vasorum.
- h.*—Elastic fibres in adventitia, cut across.

Fig. 61.

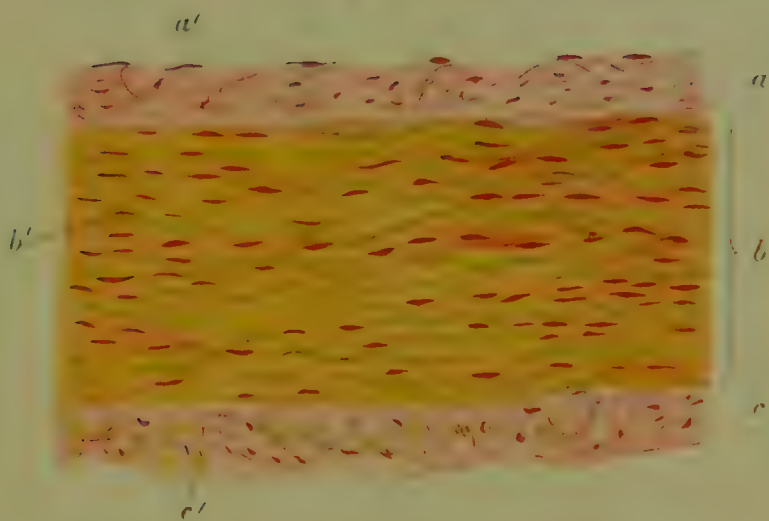
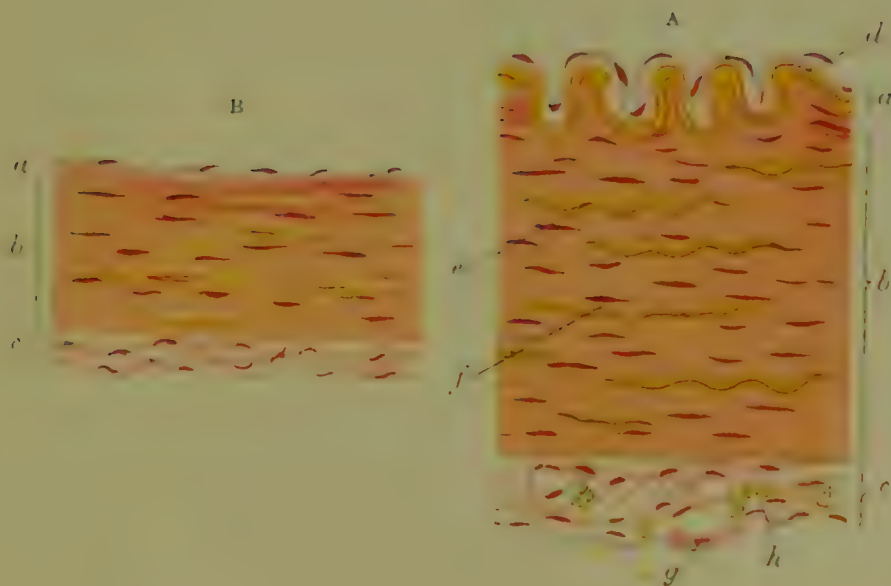


Fig. 62.



vessels supplying nutriment to the artery itself, are found. They send branches into the outer part of the muscular coat, which is, therefore, unlike the intima, not wholly extra-vascular. Nerve fibres, also of the non-medullated variety, are found in considerable numbers in the outer coat, whence they pass to the muscle elements of the media, in which they terminate.

Structure of the Aorta.—The aorta differs from such an artery in the following particulars:—

The intima shows a considerable amount of sub-endothelial connective tissue of the white fibrous variety, with longitudinally running networks of elastic fibres. There is no internal elastic lamina, distinct from the plates of the media.

The media shows a much greater proportion of elastic plates and white fibrous tissue than is to be seen in the middle coat of a medium-sized artery.

If an artery be traced down to its subdivision into capillaries, the various coats disappear in the following order:—

First, the sub-endothelial connective tissue, with the exception of the internal elastic lamina; secondly, the muscular coat, which is represented ultimately by a few isolated muscular fibres, arranged circularly or spirally around the internal elastic lamina, and then disappears; thirdly, the internal elastic lamina, leaving merely the epithelium and the remains of the adventitia.

It is difficult to say at what stage this may disappear as a coat, because the capillaries themselves, consisting merely of epithelium, usually lie surrounded with a certain quantity of connective tissue.

Structure of Capillaries.—A capillary blood-vessel is a tube of epithelial cells, cemented together by their edges, resembling in form and arrangement those lining an artery. They are flattened, nucleated, fusiform, with their long axis placed in that of the capillary itself.

Structure of the Veins.—The veins resemble the arteries in structure. In general, they may be said to have a larger lumen and thinner walls than the arterial branches to which they correspond. Their coats contain a greater proportionate amount of white fibrous, and a smaller of yellow elastic tissue, than do those of the arteries. They also contain less muscle.

The epithelium lining a vein differs in outline from that of an artery. The cells are shorter and broader, but, otherwise, they are similar.

The sub-endothelial tissue, if present, is represented by a narrow layer, containing both white and elastic fibres. There is no distinct internal elastic lamina.

The media, a much thinner coat than that found in arteries, consists of non-striated muscular fibres, white fibrous tissue, and a small proportion of elastic fibres. The definite elastic plates, seen in the media of arteries, are not present.

The veins, in different situations, vary very much as to the amount of muscular and elastic tissue present. In many, it is difficult to distinguish between a middle and an outer coat, the two being fused together, and consisting of an admixture of muscular and connective tissue elements. The saphenous vein, or the brachial, may be taken as fairly typical. Those of the brain, the retina, the corpora cavernosa, and bones, are without muscular fibres; the portal veins, and those of the uterus during gestation, are very muscular; and those of the extremities show a considerable elastic element.

Most veins, especially those of the limbs, have valves. These are composed of a prolongation inwards of the intima of the vessel, corresponding, morphologically, with the valves of the heart. Some veins, such as the intra-cranial and pulmonary, those of many of the abdominal viscera, and veins of less than $\frac{1}{2}$ in. diameter, have no valves.

Examine the following specimens :—

(1.) *V.S. Left ventricle of human heart stained with picrocarmine or hæmatoxylin. F. or B. (Figs. 48 and 52).*

First, examine with the low power, and determine the position of the peri- and endocardium.

Look for the pericardium. It is much thicker than the endocardium, and its thickness may be greatly exaggerated by the presence of fat cells in the deeper part of it. It will be recollected that, normally, there is a certain amount of fat filling the furrows upon the surface of the heart, and this may be increased to such an extent as to almost envelop the entire organ. When present in any quantity, it is, therefore, an easy matter to distinguish the peri- from the endocardium under the microscope. The endocardial surface of the heart is more or less irregular as compared with the pericardial, owing to the presence of the *columnæ carneæ*, which give rise to irregularities on its inner surface. Even under this power, it can be observed that the muscular fibres, *i.e.*, those of the *columnæ carneæ* and *musculi*

papillares, immediately below the endocardium, are frequently cut transversely. The fibres in the rest of the myocardium are cut in various directions, often longitudinally or obliquely. Look for strands of connective tissue penetrating the myocardium from the pericardium, on the one hand, and the endocardium on the other.

Under the high power, study first the pericardium. Notice that it is composed of ordinary connective tissue, *i.e.*, white fibrous tissue, with a certain amount of elastic tissue. The elastic fibres, when cut across, appear in a picro-carmin stained specimen as yellow refractile dots, which stand out in contrast with the pink of the gelatigenous element. The carmine stained nuclei of the connective tissue corpuscles are easily seen. Now look for the epithelial lining on the surface. This is liable to be destroyed during the preparation of the tissue for hardening, from the delicacy of the cells, but if it has been preserved, all that can usually be seen in such a section, are the flattened nuclei, the cell plates forming merely a line in section. Look for capillary blood-vessels, nerves, and lymphatic spaces. Find a prolongation of the pericardium, passing into the myocardium; trace it inwards, and observe how it gradually fades away into the connective tissue between the individual fibres themselves. Study the myocardium. First examine a portion of the section showing the muscle fibres, cut for the most part longitudinally (*Fig. 52*). Observe the characters of the muscle cells; short; cylindrical; and branching; with transverse and longitudinal striation, of which the former is the more marked; and the nucleus in the centre of each. Look for serrated lines (*d*) passing transversely across the fibres at recurring intervals, or across their branches. They indicate the junctions between the segments of muscle fibres, *i.e.*, between the muscle cells, or their branches. Between the fibres of the muscular network, observe the delicate connective tissue (*c*). Now examine a portion of the section in which the fibres are cut transversely (*Fig. 48*). The part where these are best seen is usually immediately beneath the endocardium, where it covers one of the muscular ridges on the inner surface of the heart, which has been cut transversely to its long axis. Notice the shape of the fibres; some round, others oval, or even polygonal, and containing a nucleus in the centre, if the section happens to have passed through the cell at the right level (*a*), or without

FIG. 63.

S. HUMAN HEART, THROUGH SINUS OF VALSALVA, STAINED WITH
Picro-CARMINE $\times 20$.

- A.—Heart wall.
- B.—Aorta.
- C.—Semilunar valve.
 - d.*—Fibrous ring in section.
 - e.*—Intima of aorta.
 - f.*—Muscular fibres.
 - g.*—Fat cells.
 - h.*—Endothelial lining.
 - i.*—Endocardium.

FIG. 64.

SMALL ARTERY IN MEMBRANE (PIA MATER OF SHEEP), STAINED
WITH HÆMATOXYLIN AND EOSIN $\times 250$.

- a.*—Adventitia.
- b.*—Muscle cell of media.
- c.*—Internal elastic lamina of intima.
- d.*—Epithelium.
- d.*¹—Nuclei of epithelium.
- e.*—Smaller artery.
- f.*—Capillary.

Fig. 63.



Fig. 64.



one, if it has passed to one side of it (*b*). Many of the transversely cut fibres show no nucleus, and are much smaller than the rest, in which case they are probably sections of the branches. Select one of the larger cross sections and examine it with the draw-tube out, in order to make out a peculiarity of cardiac muscle fibres in transverse section. With a little care in focusing, it may easily be seen that the border of the cell presents an appearance such as is represented in the figure, as though longitudinal cleavage had commenced at the periphery, and then ceased, the greater part of the cell being unaffected.

Note the thin layer of endocardium (*c*), and endeavour to distinguish the nuclei of the endothelial lining.

(2,) *V.S. Ventricular wall of heart of sheep, stained with picro-carminé. F.*

In the hearts of some ruminants, such as the ox and sheep, small collections of cells (Purkinje's) occur here and there beneath the endocardial lining of the muscular wall; if a sheep's ventricle be examined, these collections are readily found. The cells are large, nucleated, and polygonal in shape. They sometimes possess two nuclei, or a nucleus undergoing the process of division. The periphery of each shows striation.

The exact significance of Purkinje's fibres, or cells, is not known. They have the appearance of muscle cells arrested in a certain stage of development, but having, however, grown to a larger size.

(3,) *Muscle cells of human heart isolated, teased preparation, stained with picro-carminé. F.*

To see the muscle cells of the heart in a more isolated condition, tease a small piece of the substance of the wall of the ventricle (previously prepared) in picro-carminé, and mount in Farrant's solution.

Observe the more or less quadrilateral blocks into which the heart muscle is broken up. Notice the serrated appearance of the end of a cell when separation has occurred at its point of junction with another. Observe the nuclei, one to each muscle cell. The specimen improves with keeping, as the muscle takes up any superfluous stain.

(4,) *V.L.S. Cardio-aortic junction, stained with picro-carminé. F. (Figs. 61 and 63.)*

The valves of the heart may be examined in a vertical section of the junction of the aorta with the ventricle of a human heart.

A tri-radiate specimen is obtained, showing the heart wall, the aorta, and the valve, with the fibrous ring to which all three are attached.

First, note the appearance of the specimen with the naked eye, and identify the three parts referred to. (*Fig. 63.*) The broad triangular portion (A) belongs to the ventricle, and from it the riband-like section of the aorta (B) springs; from the junction of these two proceeds the cusp of the valve (C) a much narrower process. Now examine with the low power. Recognise the pink fibrous tissue, forming a common point of attachment for the three divisions. This fibrous tissue (*d*) is a transverse vertical section of the fibrous ring found at the aortic opening, to which the vessel, the wall of the heart, and the cusps of the valves are united. Trace the pink fibrous tissue, first amongst the variously cut bundles of muscular fibres in the broader or cardiac portion of the specimen. Observe the contrast between the pink stain and the browner colour of the muscle cells (*f*). Find the endocardium (*i*): trace it towards the junction where it fuses with the rest of the fibrous tissue at this point; and on into the valve (C), noticing that the latter is merely a prolongation of it. Start again from the place of junction, and trace the fibrous tissue into the aorta, where it becomes continuous with the rather broad intima (*e*). Under the high power, determine the more minute structure of the parts; study the valve, composed of white fibrous tissue, with here and there yellow elastic fibres, which, when cut across, appear as bright refractile points; the nuclei of the connective tissue corpuscles deeply stained with the carmine; and, lying on the surface, the nuclei of the epithelial lining (*h*). These are not always to be easily made out, but usually, with a little care, they can be seen as small, deeply stained, flattened bodies, closely applied to the surface.

Study the aorta (*Fig. 61*). Note the structure of the intima (*a*); the absence of a distinct internal elastic lamina; and the presence of many elastic plates (*b'*) in the media (*b*); as though the absence of the former had been compensated for by the presence of additional elastic plates in the latter.

(5) *T.S. Radial artery, human, stained with picro-carmine. F. (Figs. 60 and 62.)*

Under the low power (*Fig. 60*), recognise the three coats, and observe their relative breadth. First, find the internal elastic lamina (*b*). It stands out as a bright refractile band, stained

with the picric acid, and indicates the line of junction of the intima and media. It is very easily recognised, through the difference between its refractive index and that of the tissue around it, its very sinuous convoluted course, and its action towards reagents. With a higher power (*Fig. 62 A*), look for the rest of the intima (*a*) within the internal elastic lamina (*d*); note its proportionate thinness, compared with the intima of the aorta. Here, the amount of sub-endothelial connective tissue between the epithelium lining the vessel and the elastic membrane is comparatively insignificant. Observe the broad muscular coat (*b*) with its elongated rod-shaped nuclei (*f*), and the sinuous refractile appearance of the sections of the elastic plates (*e*); the adventitia containing the *vasa vasorum* (*g*). In the adventitia (*c*), look for the transverse sections (*h*) of longitudinally running elastic fibres, especially where it joins the media.

(6.) *T.S. Brachial vein, human, stained with picro-carmine. F. (Fig. 62, B.)*

Examine the vein with both high and low powers, observing, especially, the narrowness of the muscular coat as compared with that of the artery, and the absence of an internal elastic lamina. Note also the comparatively small amount of elastic tissue in the media, and the increase in white fibrous tissue. Frequently, no very distinct line of separation is to be made out between the media and adventitia.

(7.) *Rabbit's intestine (muscular coat) injected with nitrate of silver. B. (Fig. 10, page 92.)*

Inject a rabbit's aorta with one quarter per cent. nitrate of silver solution, until the fluid has thoroughly circulated through the systemic vessels; harden in spirit in a situation exposed to diffuse daylight. A small part of the intestinal wall is taken; the mucous membrane scraped off with a scalpel; the remaining muscular coat thoroughly dehydrated with alcohol, cleared in oil of cloves, and mounted in balsam. The successful preparation of the specimen depends upon the care with which the injection has been originally made (*see* page 121), and also upon the thoroughness of the process of dehydration and clearing, which the tissue has subsequently undergone. As usual, when specimens more difficult to dehydrate and clear than ordinary sections are to be mounted in balsam, it is well to carry out the two processes in watch-glasses, rather than on the slide. In the latter case, there

FIG. 65.

CENTRAL TENDON OF DIAPHRAGM OF GUINEA PIG, STAINED WITH
NITRATE OF SILVER $\times 300$.

- a.*—Cell space.
- b.*—Intercellular matrix.
- c.*—Lymph capillary.

FIG. 66.

PERIVASCULAR LYMPHATIC, STAINED WITH NITRATE OF SILVER AND
HÆMATOXYLIN $\times 300$.

- A.—Small artery.
- B.—Lymph space.

Fig. 65.

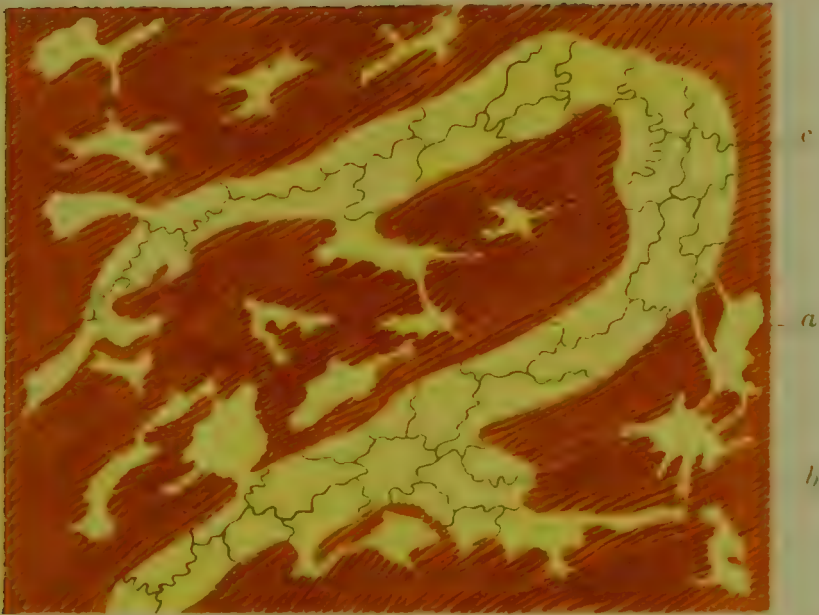
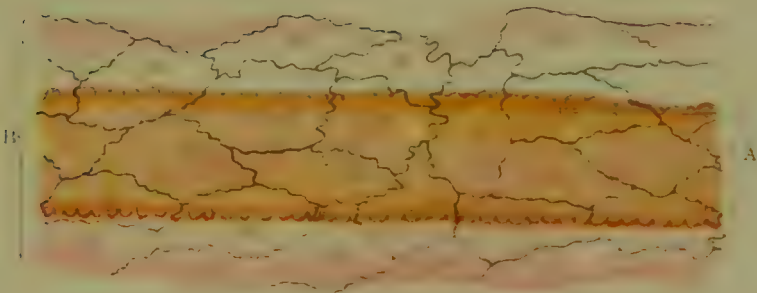


Fig. 66.



is great danger of insufficient dehydration and clearing, leading to the formation of an emulsion when the balsam is added.

Observe the arterial, venous, and capillary network (*a*, *b*, and *c*). The arteries and veins are usually found lying side by side, and giving off corresponding branches in their course. The veins are the broadest. The epithelial cells lining the vessels have their outlines revealed by the nitrate of silver stain, which is reduced by the cement substance uniting their edges. Compare the shape of an epithelial cell in an artery, with one in a vein, noting the greater breadth and less length, in the latter. *Fig. 8 A* shows the epithelium of an artery under a high power.

Look for a lymphatic vessel (*d*). It may be recognised from the fact that its contour, unlike that of the blood-vessels, is not even, but shows alternate bulgings and constrictions, and also by the very sinuous outline of the epithelial cells forming its wall.

The cement substance between the muscular fibres, forming the media of the arteries and veins, reduces nitrate of silver, and gives to the vessels the transverse lines which are so beautifully seen in such a specimen. Observe that these lines are absent from the walls of the capillaries and lymphatics. The system of parallel lines, crossing the field generally, is due to reduction of the salt by the cement substance between the muscular fibres of the wall of the intestine itself. As the external and internal coats are longitudinal and transverse respectively, systems of lines at different foci are to be seen crossing each other at right angles.

(8,) *Pia mater of sheep, stained with hæmatoxylin and eosin. F. or B. (Fig. 64.)*

Dissect from the surface of a recently obtained sheep's brain, a small piece of the closely adhering pia mater; harden for twenty-four hours in spirit and water; stain in hæmatoxylin or carmine. Mount in Farrant or balsam.

The pia mater is a membrane composed of connective tissue, closely investing the convolutions of the brain, and richly supplied with blood-vessels.

Examine with the low power, and notice arteries, veins, and capillaries traversing every part of the specimen. Select one of the arteries, and put on the high power. Do not select too large a vessel, but rather, one about to break up into capillaries, in order that the transition may be seen. Notice the elastic lamina

(*c*), as a thin, clear line bounding the lumen, acting as a basement membrane for the epithelium (*d*). Now look for transversely placed, non-striated muscle fibres (*b*). These may be seen crossing the vessel, their nuclei lying also transversely to its long axis. Within the lumen may be seen oval-shaped nuclei (*d'*), their long diameter in the axis of the tube; these are the nuclei of the epithelial lining.

Now trace the artery towards its termination, that is, the point at which it breaks into capillary branches. Observe that the muscular fibre-cells are seen to become more and more isolated as the course of the vessel is followed, until, at the capillary, they disappear altogether. The internal elastic lamina also vanishes, and the epithelium alone is left, invested by a small amount of delicate connective tissue, representing the adventitia.

THE LYMPHATIC SYSTEM.

The tissues of the body are bathed with a fluid called lymph, practically identical with the plasma of the blood, except that the proportion of water to solids, in the former, is a good deal greater. The lymph coming from the intestine during digestion contains, in addition, a considerable quantity of fat. The lymph from all parts of the body finds its way into the blood-stream at the junction of the subclavian and jugular veins in the neck, and, as a fluid distinct from the blood, it may here be regarded as lost. The mixture of blood and lymph passes then to the right side of the heart, from here to the left, through the lesser or pulmonary circulation, and from the left side to the capillaries in the tissues, by the greater or systemic arterial tree. It exudes through the capillary walls into the lymph spaces in the connective tissue in which they are imbedded. From these spaces it is collected into larger ones, called lymph capillaries, limited by a definite epithelial wall, and from these passes into the lymphatic vessels — tubes with a structure somewhat similar to that of veins. The lymphatic vessels convey it into the larger ducts on either side of the vertebral column, by which it is poured again into the vascular circulation at the root of the neck.

Throughout the body, lymph glands are situated here and there in the course of the lymph vessels.

ORIGIN OF THE LYMPHATICS.

When the lymph has passed through the wall of the capillary blood-vessel, it comes in contact with the elements of the connective tissue in which it lies. This tissue it thoroughly saturates. From it, it is conducted by the lymphatic vessels to the larger vertebral trunks. How is this effected? What is the path by which the lymph is collected from the saturated tissues?—in other words—What is the origin of the lymphatics?

There is little reason to doubt, that the *lymph spaces* in connective tissue may be considered their starting point. Some of these are merely cell spaces, in the strict sense of the words, *i.e.*, the space occupied by the connective tissue corpuscle in the matrix in which it lies; some are larger, as for instance, the areolæ amongst the bundles of fibres of areolar tissue, and these may show an indication of an attempt on the part of the cells claspings the bundles, to form an epithelial lining to the spaces. These spaces, whether merely the “lacunæ” of the corpuscles, or the larger areolæ, are in communication directly, or through the medium of similar spaces, with a labyrinth of passages, forming a network, termed *lymph capillaries*. The latter are large spaces in connective tissue, tubular or irregular in form, anastomosing freely with each other, lined with a layer of nucleated squamous cells with a markedly sinuous outline.

The nature of the connection between these channels and the cell spaces has been a somewhat disputed point. In a specimen of central tendon of diaphragm, stained with nitrate of silver, the fibrous matrix is stained a deep brown colour, while the cell spaces are nearly colourless, exhibit an irregular, branched form, and are in communication with each other by their processes. The lymphatic capillaries stand out from the general matrix as an unstained labyrinth enclosed by a layer of epithelial cells, whose sinuous outline is revealed by the reagent. As represented in *Fig. 65*, those cell spaces which are next to a lymph capillary, appear to be in communication with its lumen, by one or more of these processes, and this would afford a simple explanation of lymphatic origin, the cells lining the capillary being regarded as differentiated connective tissue corpuscles, and the lumen of the capillary as a differentiated connective tissue space.

The importance of this free permeation of a tissue by lymph

flowing through its system of cell spaces, is particularly obvious in tissues which are essentially extra-vascular, such as the cornea, bone, and cartilage. Although, in the latter case, the communicating channels between the cell cavities are not readily demonstrated in the human subject, they are very evident in the cartilage (hyaline) of cuttlefish, and, by analogy, one would suppose that a similar communication, though less distinct, may exist in the cartilage of mammals.

The central lacteal of a villus of the intestine may be regarded as a lymph capillary, ending blindly in the adenoid reticulum which supports it, and joining, by its proximal end, the lymph capillary network in the deeper part of the mucosa. In this case, the lymph from the surrounding blood capillary network in the villus traverses the meshes of the adenoid tissue, and passes through the epithelial wall of the lacteal, carrying with it the fat globules derived from the contents of the intestine. Here, at least, there seems to be no evidence of an anatomical communication between the lumen of the lymph capillary and the meshes or lymph spaces of the surrounding connective tissue.

Perivascular lymphatics.—Lymph capillaries are found in some situations surrounding vessels (*Fig. 66*); see "Central Nervous System."

Serous cavities.—A serous cavity may perhaps be considered a lymph space, greatly extended in order to serve certain purposes—amongst others, to permit of free, nearly frictionless movement of one surface over another. As examples, we may take the pleural and pericardial sacs, the peritoneum, the tunica vaginalis. The *synovial sheaths of tendons and bursæ* may be similarly classed, though they contain a more viscid fluid. In the case of the pleura, we have, developed in the midst of a fibrous covering, an enormous lymph space—so large, indeed, that it extends laterally till it has divided the investment into two layers—an outer, or parietal, adherent to surrounding parts; and an inner, or visceral, united to the lung itself. *Fig. 69* shows this diagrammatically. This space is lined by a layer of flattened squamous cells, which, however, are polygonal, and not sinuous in outline. The result of such a cavity in this situation is, that the lung, in expanding and contracting, can move with perfect freedom and with a minimum of friction, upon its surrounding surfaces.

What connection has this cavity with the lymph stream? It is virtually a lymph sac, surrounded on all sides by connec-

tive tissue, containing inter-communicating lymph spaces, which open into it. Everywhere over the epithelial surface we find small stomata, or openings between the cells, which lead into the lymph spaces or lymph capillaries of the fibrous tissue beneath. Thus, the pleural sac is in connection with the lymph stream in the connective tissue—on the one hand lining the cavity in which the lung is placed, and on the other investing the organ itself. This is very readily understood if it be remembered that these visceral and parietal layers of fibrous tissue are virtually one, which has been split, so to speak, into two sheets, by the development in its centre of a large lymph space.

The ordinary bursa developed in the connective tissue covering a bony tuberosity—that is to say, placed where a considerable amount of free movement is necessary between the skin and the deeper parts—is an example of the development of one of the more simple sacs, which may make the lymphatic relations of more highly specialised pleural, pericardial, or peritoneal cavities, more easy of comprehension.

In the case of a bursa, we have to deal with a sac similar to the pleura, but one which has not undergone any special modification in shape by being folded round a particular organ. It is simply a bag, lined with an incomplete layer of connective tissue cells of an epithelioid type, the wall consisting of a thin, condensed layer of fibrous tissue. When the skin is pressed upon and moved from side to side over the projection of bone which the bursa protects, the one surface of the flattened bag slips freely in any direction over the opposing one. The cavity of the bursa, however, contains, not ordinary lymph, but a viscid, glairy fluid, more fitted for the purpose of lubrication of surfaces exposed to considerable friction. This is a good illustration of specialisation of function to meet what may be regarded as virtually an accidental demand. Two walls of connective tissue rub together, and the ordinary cells of the tissue at the surface arrange themselves in the form of an epithelial lining, and in order still further to diminish friction, a synovial or viscid fluid is “secreted” into the space.

THE LYMPH VESSELS.

The lymph passes from the lymph capillaries into the smallest lymphatic vessels. These, usually smaller in lumen

FIG. 67.

DIAGRAMMATIC REPRESENTATION OF ORIGIN, COURSE, AND TERMINATION OF LYMPH CHANNELS.

- A.—Lymphatics of limb.
- B.— „ „ intestine.
- C.— „ „ lung.
- a.*—Vein at root of neck.
- b.*—Thoracic duct.
- c.*—Lymphatic vessels on proximal side of glands.
- d.*—Lymph glands.
- e.*—Lymphatic vessels on distal side of glands.
- f.*—Lymph capillaries.
- g.*—Lymph (cell) spaces.
- h.*—Central lacteal of villus of intestine.
- i.*—Pleural sac.
- j.*—Blood-supply to lymph glands.

FIG. 68.

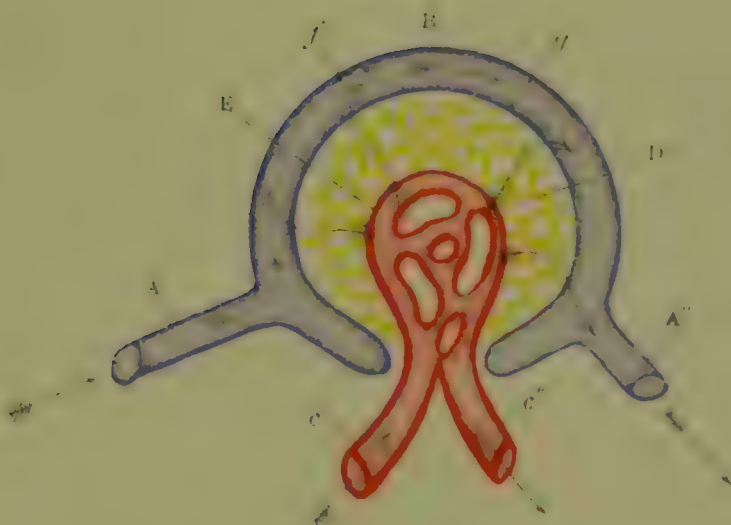
DIAGRAMMATIC REPRESENTATION OF SOLITARY FOLLICLE.

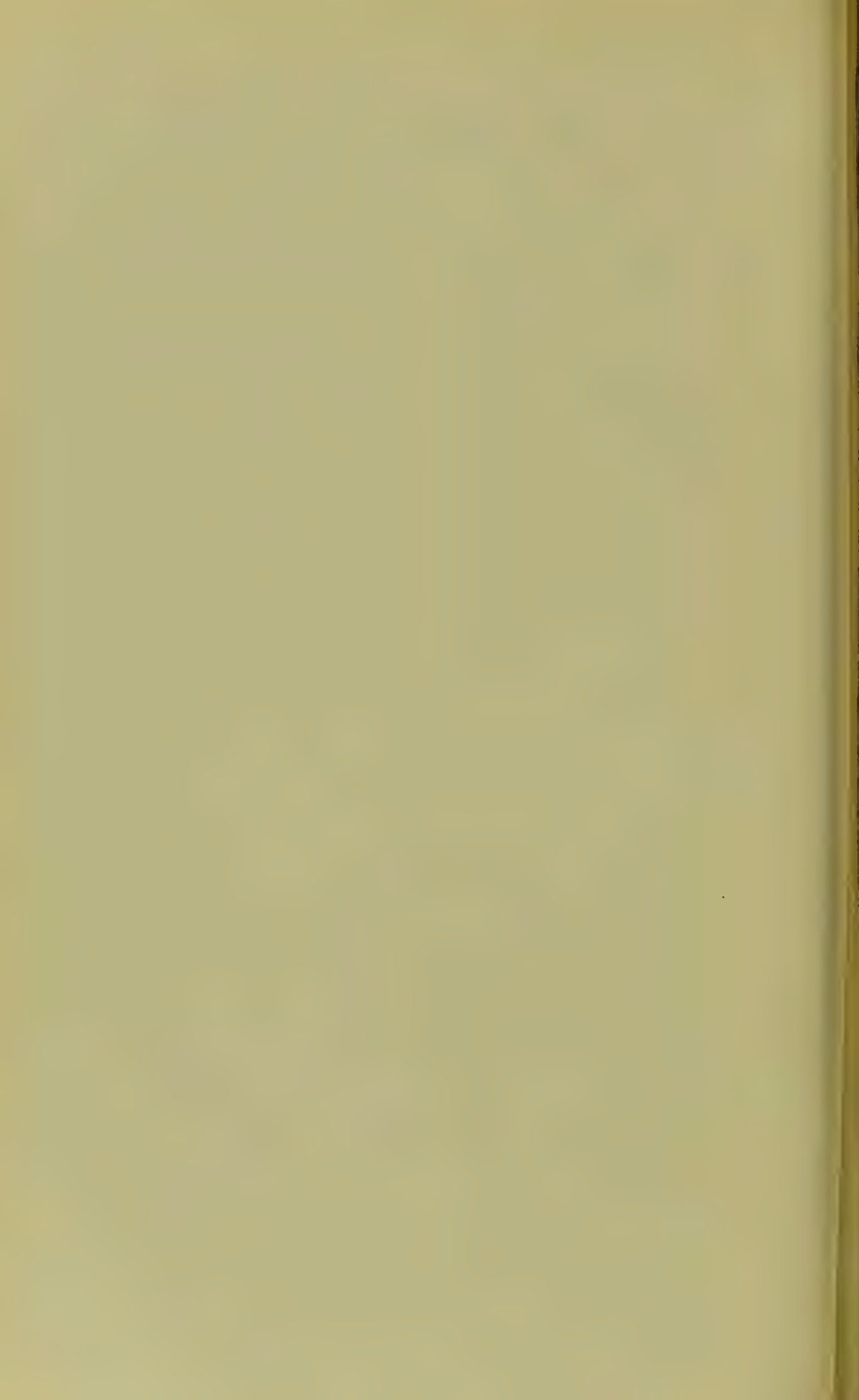
- A.ⁱ—Afferent lymph vessel.
- A.ⁱⁱ—Efferent „
- B.—Sinus surrounding follicle.
- D.—Adenoid tissue.
- E.—Blood capillary network.
- c*ⁱ.—Afferent blood-vessel.
- c*ⁱⁱ.—Efferent „
- f.*—Outer wall of sinus.
- g.*—Inner wall.

Fig. 67.



Fig. 68.





than the capillaries from which they spring, differ from them in being lined with fusiform, epithelial plates, not distinctly sinuous in outline, and in possessing valves. Beyond the epithelium, there is a very thin connective tissue investment. They anastomose freely with each other, and often present a beaded appearance, due to dilatation on the proximal side—that is, the side nearer the heart—of the valves.

From these smaller vessels, the larger ones, conveying the lymph towards the thoracic duct, take origin. They are very similar in structure to veins, and need not, therefore, be described in further detail. They possess numerous valves, and, owing to the thinness of their walls, tend to show bulgings on the proximal side.

The thoracic duct is also similar to a vein. It is, however, more muscular than elastic, owing to the small amount of connective tissue in its walls, and to this is probably due its greater friability (Foster). It does not, however, progressively widen as it approaches its termination in the neck, being broader at its lower end, where it receives the fat from the intestine, and is termed the *receptaculum chyli*. It has numerous valves.

THE SOLITARY FOLLICLES.

In some of the mucous membranes of the body, solitary follicles frequently occur in the course of the smaller lymphatic vessels. In the small and large intestines, they occur as small rounded bodies, about the size of a pin's head, partly in the mucous, and partly in the sub-mucous coat. They are found also as *Peyer's patches* (agminated patches or collections of follicles) in the small intestine opposite the line of its attached or mesenteric border, especially in the ileum. Solitary follicles are also found in the sub-mucosa of the bronchi and bronchioles, in the tonsils, etc.

Structure.—A solitary follicle is a more or less rounded mass of adenoid (or lymphoid) tissue, supporting a network of capillary blood-vessels, the whole surrounded by a lymph sinus in communication with the neighbouring lymphatic vessels. The lymph sinus is lined with a layer of epithelial cells with a sinuous outline. It is interrupted here and there with strands of connective tissue, passing between the adenoid reticulum within the follicle and the connective tissue outside. Some of these strands,

or bridles, contain blood-vessels, bringing blood from without to the capillary network, or carrying it in the opposite direction. Each strand is covered with epithelium, continuous with that lining the walls of the sinus through which it passes.

The adenoid tissue, forming the mass of the follicle, consists of the usual delicate network of fibrils invested with branched epithelioid cells. The meshes of the reticulum are filled with lymph corpuscles, small round cells, with a single large nucleus, and very little perinuclear protoplasm. So completely do the lymph corpuscles preponderate that they almost entirely obscure the adenoid network.

At the periphery of the rounded mass, the adenoid reticulum or, more properly speaking, the epithelium covering it, is continuous with that of the inner wall of the lymph sinus, the meshes of the network probably communicating with this space through imperfections in the continuity of its inner epithelial wall.

If we trace the course of the lymph from the blood capillaries, we find it first permeating the meshes or cell spaces of the adenoid tissue, whence it passes into the lymph sinus, by the path just indicated. From the lymph sinus it passes into the lymphatic vessels, in the course of which the follicle occurs, and so away into the general lymph stream (*Fig. 68*).

We see thus, that we have here to deal with a somewhat similar arrangement to that obtaining in ordinary areolar tissue ; in fact, merely a specialisation of it. The following comparison will show the relationship more clearly :—

Areolar or ordinary connective tissue.		Lymph follicles.	
Fibrous tissue	=	Fibrils of adenoid reticulum	
Connective tissue cells	=	Epithelium of reticulum	
Cell spaces	=	Meshes of network	
Lymph capillary	=	Lymph sinus	
Lymph vessels	=	Lymph vessels	
Leucocytes	=	Leucocytes in greater number	
Mucin matrix	=	(Absent)	

A Peyer's patch is merely an aggregation of solitary follicles, and need not, therefore, be referred to in greater detail.

THE LYMPHATIC GLANDS.

These are nothing more than highly specialised lymph follicles. We have in the case of a lymphatic gland to

deal again with an afferent and efferent system of lymph vessels; an intermediate lymph sinus, lined with sinuous epithelium; and, enclosed in the sinus, a mass of lymph follicular tissue, in which is embedded a network of capillary blood-vessels. A lymph gland, however, is much larger than a solitary lymph follicle, and, on account of its size and exposed situation, it possesses a supporting framework of connective tissue, which is unnecessary in the case of smaller follicles, sufficiently protected and supported as they are, by the tissues in which they are imbedded.

The supporting framework of connective tissue takes the usual form found in other organs, such as the lung, liver, etc., of a fibrous capsule sending septa or trabeculæ into the interior, which is thus split up into a series of compartments. The lymph sinus surrounds the trabeculæ everywhere, separating them and the capsule from the lymph follicular tissue.

A lymphatic gland is somewhat reniform in shape, and varies considerably in size, even in the same animal. It possesses a hilum at the side where the artery enters it, and the veins and efferent lymphatic vessels leave it. The afferent lymphatic vessels enter at various points of its periphery. The gland consists of two parts, the cortex and the medulla. The former lines the capsule, except at the hilum, where the medullary or central part reaches the surface. *The capsule* consists of two layers of fibrous tissue, an outer coarser, and an inner finer one, between which run the lymphatic afferent vessels, in the form of a network, before they open into the lymph sinus beneath. From the under surface of the capsule are sent *septa*, or *trabeculæ*, directed towards the centre of the gland. In many animals, both the capsule and the trabeculæ contain non-striped muscular fibres. These trabeculæ divide the cortex into segments of the shape of a truncated pyramid, the base of which is directed to the periphery. The central, or main part of each of these segments, consists of a *lymph follicular mass*—a lymph follicle in fact; and between it, and the trabeculæ at its sides and the capsule at its base, is the lymph sinus. At the junction of the medullary part of the gland and the cortical or peripheric, the septa or trabeculæ begin to branch and anastomose, with the result that the lymph follicular masses cease to possess a block-like form, and are cut up to form in the medulla a network of what are

FIG. 69.

DIAGRAMMATIC REPRESENTATION OF SEROUS CAVITY.

- A.—Solid capsule of connective tissue investing bone, cartilage, etc.
 B.—Capsule of connective tissue, divided by lymph space into two layers,
 parietal and visceral, investing such an organ as the lung.
 a.—Parietal layer.
 b.—Visceral „
 c.—Lymphatic space between.
 d.—Root or peduncle of organ.
 E.—Organ itself.

FIG. 70.

SECTION OF LYMPHATIC GLAND OF SHEEP, STAINED WITH HÆMA-
TOXYLIN × 20.

- a.*—Capsule.
b.—Trabeculæ.
c.—Cortex.
d.—Medulla.
e.—Hilum.
f.—Blood-vessels cut transversely.
g.—Lymph sinus.
h.—Lymph follicular tissue.

Fig. 69.

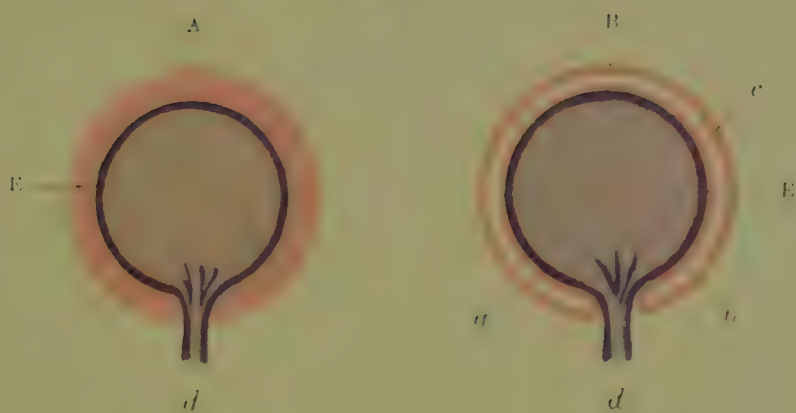
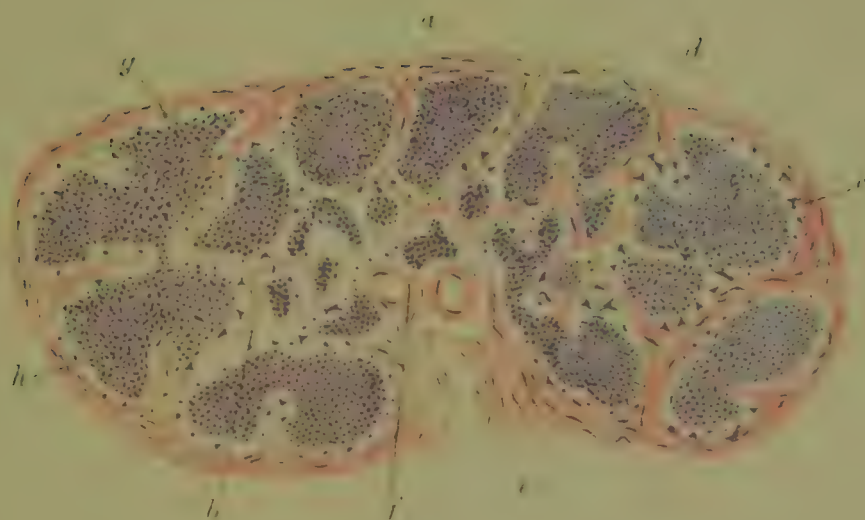


Fig. 70.



termed *lymph follicular cords*. Here, as in the cortex, the lymph sinus separates the septa and the lymph follicular tissue. The fibrous tissue framework, the lymphoid tissue, and the intervening sinus, form three distinct systems, continuous in themselves, throughout the gland.

The lymph sinus is very similar to that surrounding a solitary follicle, but differs from it in being traversed by a reticulum of adenoid tissue. It differs, however, from adenoid tissue in other situations, in the comparative coarseness of the network, and in the small number of leucocytes, or lymph corpuscles, found in its meshes. These are so small in number, that if a section be pencilled with a camel's hair brush, or shaken to and fro in water, they are easily removed from their position, and the adenoid network clearly revealed. On this account, the sinus of a lymph gland is usually selected to demonstrate the nature of adenoid tissue.

The lymph follicular tissue, both in the cortex and medulla, is, in every respect, similar to that found in a solitary follicle. The lymph sinus is lined throughout with a layer of squamous epithelial cells with a sinuous outline, applied on the one hand to the surface of the trabeculæ, and on the other, to the surface of the lymphoid tissue. It is continuous between with the epithelium covering the adenoid fibrillar network and the larger strands of connective tissue, supporting blood-vessels, which traverse the space.

Course of the blood stream through the gland.—The afferent blood-vessel enters at the hilum and, passing into the trabecular system, is thus distributed to every part of the organ. At intervals, branches leave the trabeculæ, traverse the lymph sinus (in which they are covered with epithelium continuous with that lining its wall) and enter the lymph follicular tissue, in which they break up into capillary networks. From the network emerge veins which traverse the lymph space in the reverse direction and, accompanying the arteries in the fibrous tissue framework, find exit from the gland at its hilum.

Course of the lymph stream through the gland.—The afferent lymph vessels pour their contents into the sinus beneath the capsule, after forming a plexus between its two layers. It flows along the sinus (where it is augmented with that derived from the capillaries in the lymph follicular tissue) and leaves the organ by the efferent lymphatic vessels passing out at the hilum.

Functions of lymph follicles and lymphatic glands.—One of the chief functions of lymphoid tissue is the production of lymph corpuscles or leucocytes. In lymphatic glands, the follicular masses often present a light central area, which does not readily stain with reagents, and a more deeply stained periphery. When the cells towards the centre of the mass are examined under a high power, they are found to be larger than those further out, owing to an increase in the amount of perinuclear protoplasm, which is very lightly stained. Many of these cells show mitotic changes in their nuclei. The lymph leaving the lymph glands contains a greater number of corpuscles than that going to them.

Lymph glands also serve as sieves for the arrest of foreign particles contained in the lymph passing through them. Thus, pigment, injected into the loose tissue of the hand, is arrested in those lymph glands through which it has to pass on its way through the lymph vessels to the trunk. In the case of a dissecting wound, the glands are early affected owing to their attempt to arrest and destroy the microbes in the lymph passing through them.

But, in addition to these two functions, there is, doubtless, a continuous process of interchange between the blood and the lymph flowing through the gland, though we do not yet know definitely its exact nature.

Fig. 67 shows, diagrammatically, the more important lymph connections in the body. (A) may be taken as representing the lymphatic connections in the arm. The cell spaces—the starting point of the lymph channel—are represented at (*g*), and the lymph capillary system into which they open at (*f*). From these the lymph is conveyed by the lymph vessels (*e*) to the lymph glands (*d*), and thence by the lymph vessels (*c*) to the thoracic duct (*b*). (B) represents the course of the lymph coming from the small intestine; (*h*) is the central lacteal of a villus joining (*f*) the labyrinth of lymph capillaries in the deeper part of the intestinal wall. From this plexus lymph vessels (*e*) proceed to the mesenteric glands (*d*), and from them again vessels (*c*) opening into the thoracic duct; (C) represents the lymphatic system of the lungs. The cell spaces of the pleura and connective tissue framework are shown at (*g*); (*i*) represents the pleural sac (a large lymph capillary), and (*f*) the lymph

capillaries of the lung generally. From these, the lymph proceeds by the vessels (*e*) to the bronchial glands (*d*), and thence by the vessels (*c*) to the thoracic duct.

Fig. 69 shows, diagrammatically, the nature of the serous sac, such as that enveloping the lung. (A) represents such a structure as bone or cartilage invested with an undivided membrane (the periosteum or perichondrium), in which, of course, occur numerous cell spaces and lymph capillaries of the usual size; (B) represents the lung in which the fibrous investment has become split into two layers, an inner and outer, which are freely moveable on each other, with an enlarged lymph space between them.*

Examine the following specimens :—

(1.) *Central tendon of diaphragm of guinea-pig, stained with nitrate of silver. B. (Fig. 65.)*

The central tendon consists of a series of radiating bundles of tendinous fibres, supported by ordinary connective tissue. It is covered on each surface with serous epithelium, continuous on the one side with that lining the thoracic cavity generally, and on the other with the peritoneal. In the specimen to be examined, the epithelium of the surfaces should have been to a large extent removed by pencilling.

With the low power, observe first the network of lymph capillaries forming an irregular labyrinth. Note the varying diameter of the capillaries, which are almost unstained in contrast to the fibrous tissue in which they lie, which is coloured deep brown by the silver nitrate. Observe everywhere the small branching cell spaces, which are also unstained. Here and there are to be seen patches of polygonal squamous cells, their outlines revealed by the reagent—cells lining the serous cavity on either side of the tendon, which have escaped removal by the pencilling. Even with the low power, the sinuous outlines of the cells lining the lymph capillary labyrinth may here and there be seen. Select a part of the labyrinth showing these cells, and also with the cell spaces in its neighbourhood clearly revealed, and put on the high power. Look for an appearance

*The lung is not in reality surrounded by a fibrous covering which *subsequently* becomes split. The student of embryology will remember that the splitting of the mesoblast to form the pleuro-peritoneal cavity takes place before the development of the organs.

similar to that represented in the figure. A little confusion is liable to arise when some of the serous epithelium, covering either side of the tendon, has been left, and intervenes between the observer and the tissues it covers. A little care will, however, enable the student to differentiate it from the epithelium of the lymph capillaries. The cells of the latter are, of course, sinuous in outline, and are definitely limited to the lymph channel of which they form the lining. Those of the former, on the other hand, are polygonal in shape, and cover, not only the lymphatic network, but the tissue with its cell spaces between. The two epithelia again occur on a different level, the serous epithelium being at a higher, or lower, focus than that lining the lymph capillaries.

Note the cell spaces, irregular in shape and branched, communicating with each other and, when in its near vicinity, with the lymph capillary.

(2.) *Section of lymph gland of mammal, stained with hæmatoxylin or picro-carmin.* B. or F. (Figs. 70, 71 and 72.)

With the low power (*Fig. 70*), identify the general arrangement of parts in the gland. Find first the capsule or fibrous investment (*a*), and trace it round till the hilum is reached, when it becomes continuous with the connective tissue (*e*) entering with the blood-vessels (*f*) and efferent lymphatics. The section, however, may not have passed through the hilum, in which case the capsule will be seen as an uninterrupted band passing round the organ. Observe the septa (*b*) coming from its deeper layer and passing towards the centre of the gland. Beneath the capsule, look for the large blocks of lymphoid tissue (*h*), constituting the cortex. These somewhat quadrangular or pyramidal masses may be cut so that their connection with the lymph follicular cords of the medulla is shown; or, the knife may have passed obliquely or transversely through them, making them appear as entirely isolated structures. Look now at the lymph sinus (*g*). It is seen as a narrow light space, between the capsule and trabeculæ or septa, on the one hand, and the lymphoid tissue on the other. The sinus will, however, be distinguished more distinctly under the higher power. Now move the section to and fro, under the microscope, and observe the general difference in appearance between the cortex and medulla. The former consists of a series of deeply stained blocks of tissue, placed side by side beneath the capsule; the

medulla, on the other hand, presents the appearance of a network and is, seemingly, comparatively lightly stained. This is due to the fact that the large lymph nodules of the cortex are represented in the medulla by a network of comparatively delicate lymph follicular cords, alternating with a corresponding network, formed by the splitting up of the trabeculæ of fibrous tissue, as they pass from the cortex into the medulla. Make out this double network with the lymph sinus, still everywhere found between the fibrous and lymphoid tissue. The lymph follicular cords present, under the low power, a somewhat granular appearance, due to the staining of the nuclei of their lymph corpuscles, and are considerably broader than the trabeculæ of fibrous tissue. Between the two, the sinus may be seen as a clear space traversed by a network of adenoid tissue. In a thin section, the adenoid reticulum stretching across the sinus may be very distinctly made out, especially in the medullary part of the gland, even with the low power. In the region of the hilum, if it is shown, look for sections of blood-vessels and lymphatics.

Find the capsule again, and put on the high power (*Fig. 71*). Observe its lamellated appearance and the lymph channels between the lamellæ. Note the flattened connective tissue cells between its fibres: here and there are to be seen blood-vessels cut in different directions, often filled with blood corpuscles, stained a greenish yellow. Follow the capsule till one of the trabeculæ (*b*) coming from it is reached. Examine the sinus (*d*) and its contents. The sinus, roughly speaking, is about the breadth of the capsule in vertical section. Study the network traversing it, stretching between the capsule or septa, and the lymph follicular tissue. This network is larger and coarser than that occurring in the lymphoid tissue, which the sinus surrounds. It consists of a reticulum of delicate fibrils, of the nature of those found in white fibrous tissue, the junctions in the fibrillar network being covered with epithelial cells, whose processes extend along the bars, and anastomose with those of adjacent cells. This epithelium is continuous on the one hand with that forming the outer wall of the sinus and covering the trabeculæ or inner surface of the capsule, and, on the other, with that forming the inner wall of the sinus, and applied to the surface of the lymph follicular tissue. The fibrillar network itself is continuous with the fibrils of the connective tissue

FIG. 71.

S. OF LYMPATHIC GLAND (CORTEX , STAINED WITH Picro-CARMINE $\times 60$.

- a.*—Capsule.
- b.*—Trabeculæ.
- c.*—Lymph follicular tissue.
- d.*—Sinus traversed with adenoid tissue.

FIG. 72.

S. OF LYMPHATIC GLAND (MEDULLARY REGION), STAINED WITH
HÆMATOXYLIN $\times 300$.

- a.*—Lymph follicular tissue.
- b.*—Trabeculæ.
- c.*—Sinus.
- d.*—Adenoid reticulum of sinus.
- e.*—Blood-vessels in lymph follicular tissue and trabeculæ.
- f.*—Lymph cells.

Fig. 71.

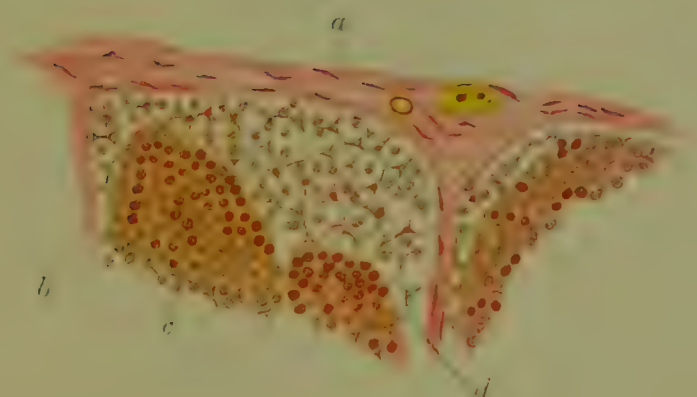
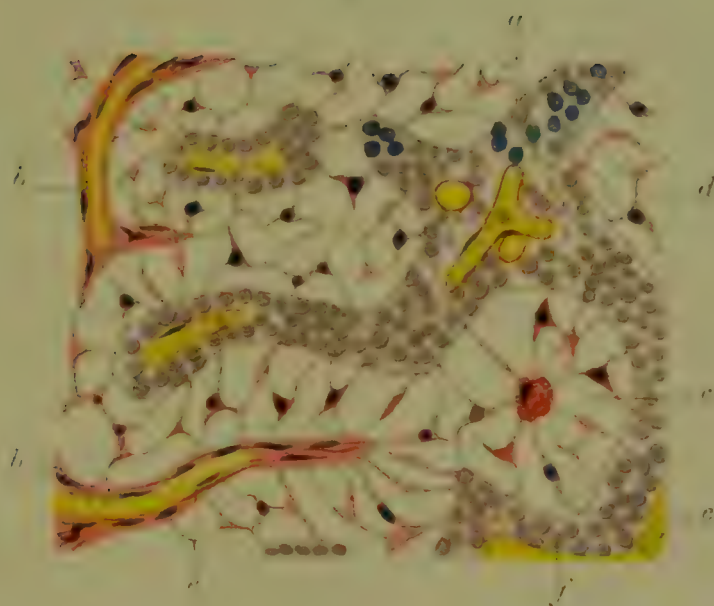


Fig. 72.



of the trabeculæ or capsule externally; and internally with fibrillar network in the lymph mass. Note the comparatively small number of leucocytes in the meshes of this network in the sinus. In fact, if the specimen has been shaken sufficiently in water previous to being mounted, all the lymph cells may have been washed away. Now study the blocks of lymphoid tissue in the cortex; and here, at first sight, little is to be seen but a dense mass of leucocytes. There are, however, some further points to be made out with more careful examination. Look at the edge of one of the masses, and distinguish the flattened nuclei, indicative of the layer of epithelial cells covering its surface. Here and there, throughout the mass, other flattened nuclei may be seen, often arranged in parallel rows in linear series. These are the nuclei of the epithelial cells, forming the walls of capillary blood-vessels. Now, find a lymph block in which the central part is less deeply stained than the peripheral, and note the change which the cells in the former have undergone. They are larger, and less affected by the staining reagent, as far as their perinuclear protoplasm is concerned. Their nuclei show mitotic changes, and the chromatic element in them is deeply stained. It is evident that in this portion of the masses, multiplication of the leucocytes is taking place. When these points have been made out, move the slide so that the medullary part of the gland comes into view. Find a portion similar to that shown in *Fig. 72*. Note again the double network of fibrous trabeculæ and lymph follicular cords. Observe the fusiform connective tissue corpuscles amongst the fibres of the trabeculæ (*b*), and frequently longitudinally or transversely divided blood-vessels (*e*). Where the bars of either network—fibrous or lymphoid—have been cut transversely, there is sometimes a little difficulty in distinguishing between the two. As a rule, however, the sections of the lymph follicular cords are broader than those of the trabeculæ of fibrous tissue, and they consist of a mass of leucocytes, which are easily recognised, when once the eye has become accustomed to their appearance. The epithelial covering of the lymphoid tissue is particularly evident in the medulla, as a definite line enveloping it with flattened nuclei, occurring at intervals. The capillaries within the cords are also more easily to be made out than they were in the lymph blocks in the cortex. Study again the network in the sinus.

A specimen of a gland may also be advantageously studied, in which the outlines of the cells of the lymph sinus have been revealed, by interstitial injection of the organ with a solution of nitrate of silver before the sections are cut. They may afterwards be stained either with hæmatoxylin or picro-carmin. Another method by which the lymph sinus may be very clearly demonstrated is to inject a gland interstitially with solution of Prussian blue (*see* "Appendix").

APPENDIX TO CHAPTER VIII.

METHODS OF PREPARATION.

1. Sections of heart wall.—Harden pieces of the left ventricle, including both peri- and endocardium in Müller, or Müller and spirit ; cut in gum, and stain with picro-carmin or hæmatoxylin ; or stain in bulk with borax-carmin, and cut in paraffin.

2. Isolated cardiac muscle cells.—Harden a piece of the heart wall in 2 per cent. bichromate of potassium for two or three days ; tease a small piece in picro-carmin ; mount in Farrant. The muscle cells do not always readily separate from each other ; but their character is more clearly seen than in a section.

3. Cardio-aortic junction.—Harden the junction in Müller and spirit ; cut and stain as for 1.

4. Arteries and veins.—Treat as for 1 and 3.

5. Muscular wall of rabbit's intestine, AgNO_3 .—(See page 121.) The intestine of a frog will do as well.

6. Central tendon of diaphragm, AgNO_3 .—(See page 47.)

7. Lymphatic gland.—Harden the lymph gland of a freshly killed animal in spirit ; cut in the long axis of the gland and through the hilum, so as to show the entrance of the blood-vessels and connective tissue. It may be cut either in gum or paraffin, and stained as for 1, 3, and 4. To see the mitotic changes in the cells of the central part of the cortical lymph masses, harden a small piece of the gland in Flemming's fluid ; cut in paraffin, and stain in saffranin.

8. Interstitial injection of lymphatic glands.—(1,) *With nitrate of silver.* With a hypodermic syringe inject $\frac{1}{4}$ per cent. solution of AgNO_3 , at random, into the central part of a fresh gland ; harden in spirit, cut in gum, and expose to diffuse daylight ; stain in picro-carmin or hæmatoxylin. The walls of the sinus are coloured brown by the reagent, the outlines of the epithelial cells lining and crossing it being revealed. (2,) *With Prussian blue.* In a similar manner inject soluble Prussian blue. Harden in spirit ; cut in gum ; stain in picro-carmin. The sinus throughout the gland is filled with the blue injection. The lymphoid tissue is stained deeply with the carmin.

CHAPTER IX.

THE SPLEEN AND "DUCTLESS GLANDS."

THE SPLEEN.

THE spleen is invested with a fibrous *capsule*, from the under surface of which are given off *trabeculæ*, which penetrate the organ, and dividing and anastomosing with each other split it up into a series of irregular compartments. These contain the parenchyma or *splenic pulp*, which, on section, presents the appearance of a dark red mass, traversed with the lighter fibrous trabeculæ, and everywhere dotted over with small round, or oval, pale bodies, about the size of a pin's head—the *Malpighian corpuscles*.

The **capsule** consists of an outer fibrous layer, covered with epithelial cells, the peritoneal investment; and an inner, also formed of connective tissue, containing non-stripped muscular fibres, which vary in quantity in different animals. The **trabeculæ** coming off from the deeper layer possess a similar structure, and are of the nature of rounded or flattened cords. They soon divide, and their divisions anastomose with each other, the same process being repeated, so that a supporting framework of fibrous tissue for the rest of the gland results. The trabeculæ contain blood-vessels, nerves, and lymphatics. The **pulp** consists of a network, analogous to that of ordinary adenoid tissue, though it possesses a distinct character of its own. It is formed of a delicate fibrillar basis, upon which an epithelial element is laid down; but these epithelial cells are larger and coarser than those of adenoid tissue generally, and are different in shape. They are large, irregular, and nucleated, with intercommunicating flange-like processes. The meshes of the network contain cells of various kinds. For the most part, they are merely red blood corpuscles, which give to the spleen pulp its chocolate colour.

when seen on section with the naked eye. Amongst the mass of red blood corpuscles are to be found a certain number of leucocytes—small round cells, similar to those found in the interstices of lymphoid tissue, having a comparatively large nucleus and very little perinuclear protoplasm. In addition are to be found other cells indistinguishable from the ordinary white blood corpuscle—amœboid cells—possessing more than one nucleus. There is, however, yet another variety of cell which is more special to this situation. It is considerably larger than a white blood cell, and possesses two, three, or more nuclei; its perinuclear protoplasm contains corpuscles indistinguishable from red blood cells, or merely pigment granules, or some material representing probably an intermediate stage between these two. The fibrous tissue of the trabeculæ is continuous with the fibrillar network of the spleen pulp; that is to say, the ultimate finer trabeculæ break up into this fibrillar network, and the larger ones give off a fringe of fibrils at their edges. The **blood-vessels** of the spleen enter and leave it at the hilum, and, as in other organs, the fibrous tissue in this neighbourhood is in excess of what it is elsewhere. The arterial branches pass into the trabeculæ, which they follow into their smaller divisions, where they leave them and plunge into the pulp substance. Here they terminate in the following manner. Their epithelial lining becomes imperfect, spaces occurring between the cells forming it, and this continues until the vessel no longer presents the form of a tube, but its lumen has become continuous with the cell spaces or meshes of the pulp tissue. The blood from these cell spaces is collected into the veins in a similar way, the cells of the pulp becoming arranged to form first a channel with perforated walls, and then a complete tube. The venules enter the trabeculæ and accompany the arteries to the hilum. Thus, we see, that between the arterioles and the venules, we have not a system of tubular capillaries, but a system of cell spaces. A comparison may be seen to exist here between the spaces of the splenic pulp opening into the arterial or venous tubes, and the cell spaces of ordinary connective tissue into a lymph capillary; the chief difference being the relative amount of connective tissue fibres present in the two cases, and the contents of the respective channels, in the one case blood, and in the other, lymph.

After the small arteries leave the trabeculæ and plunge into

FIG. 73.

S. HÆMAL GLAND OF SHEEP, STAINED WITH BORAX-CARMINE $\times 40$.

- a.*—Capsule.
- b.*—Peripheral blood sinus.
- d.*—Lymphoid tissue.
- e.*—Central blood sinus.

FIG. 74.

S. HÆMAL GLAND OF SHEEP, STAINED WITH BORAX-CARMINE $\times 350$.

- a.*—Capsule.
- b.*—Peripheral blood sinus.
- c.*—Central blood sinus.
- d.*—Lymphoid tissue.
- e.*—Adenoid reticulum in peripheral sinus.
- f.*—Lymph corpuscles (white blood corpuscles).
- g.*—Epithelial covering of lymphoid tissue.
- h.*—Blood-vessels in lymphoid tissue.

Fig. 73.

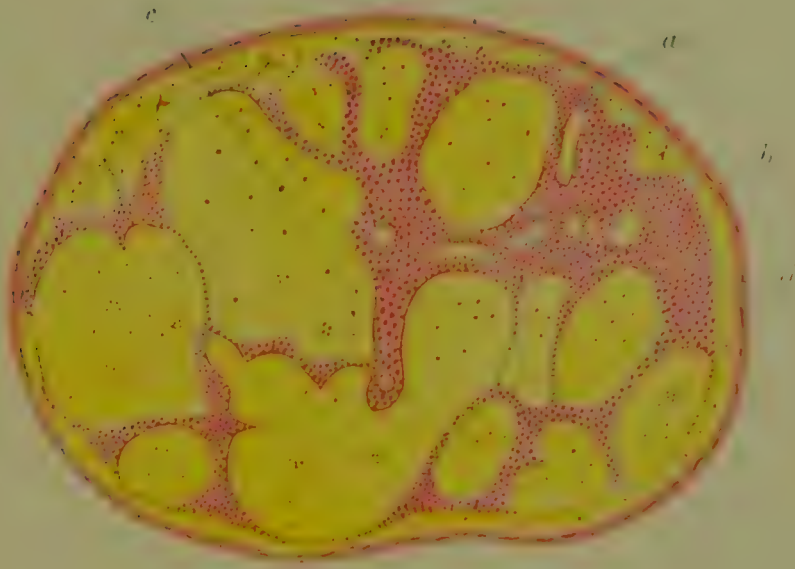
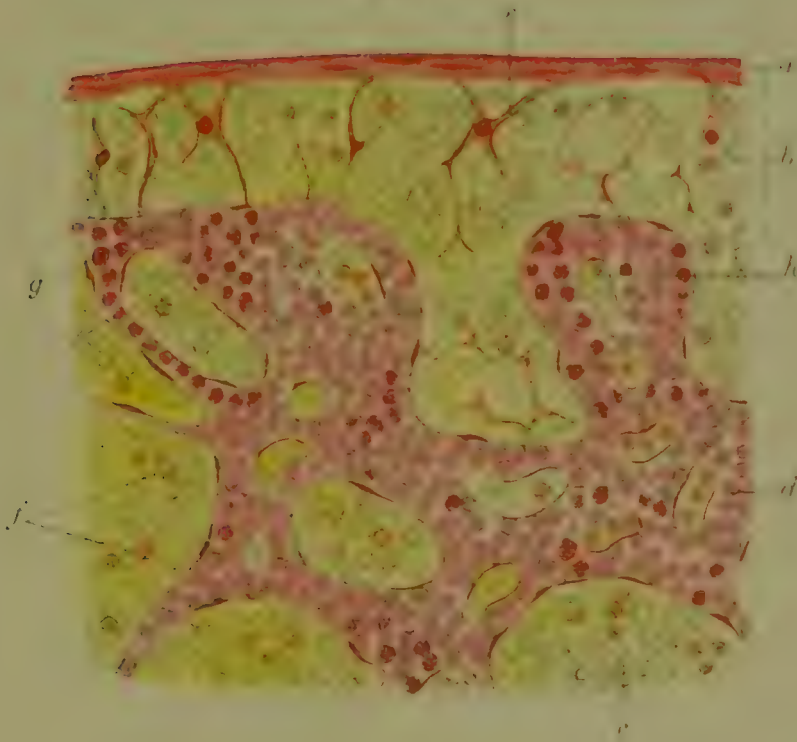


Fig. 74.



the splenic pulp, their adventitia undergoes a remarkable change at intervals. It becomes greatly increased in quantity, so as to form the small oval or spherical **Malpighian** or **splenic bodies**, about the size of a pin's head, which were seen scattered throughout the cut surface of the spleen with the naked eye. A Malpighian corpuscle is of the nature of adenoid or lymphoid tissue; and its lymph spaces are continuous with the lymphatics of the trabeculæ from which the artery has emerged. It is traversed by capillary blood-vessels, which are given off from the artery as it passes through the lymph mass, and these capillaries at its margin open directly into the cell spaces of the splenic pulp.

These Malpighian bodies often occur at the point of division of a small artery. The arteriole they invest is sometimes placed in the centre, sometimes excentrically.

The general arrangement of parts in the spleen may be studied in that of the cat.

(1,) *S. Spleen of cat, stained with hæmatoxylin. B. (Fig. 77.)*

Under the low power observe that the main part of the organ appears to consist of a blue, granular looking mass, the *spleen pulp* (*b*), and amongst this we have scattered here and there, round or oval masses of more deeply stained granular tissue, the *splenic corpuscles* or *bodies* (*c*). In the centre of each of these is sometimes to be seen a lighter area, similar to that seen in the lymph nodules of a lymphatic gland, the *germ-centre* of Flemming. Even with the low power it is quite easy to make out in the splenic body the artery (*d*) upon which it occurs, placed usually excentrically, and cut either transversely, obliquely, or longitudinally. These lymph nodules often occur at the point of division of a vessel, so that we sometimes find in one nodule the transverse sections of two vessels. Look for sections of the trabeculæ (stained pink) in the pulp, and surrounding the gland note the capsule or general investment (*a*) with which the peripheral trabeculæ are continuous.

With the high power note the rod-shaped nuclei of the smooth muscle in the capsule and trabeculæ; the eccentrically placed blood-vessel in the Malpighian or splenic corpuscles; the germ-centres of Flemming; and that there is no sinus between the splenic pulp and the fibrous framework of the organ, as in the case of lymphatic glands.

The more minute structure is best studied in a sheep's spleen,

which has been thoroughly "washed," to remove all the blood from the blood-vessels, and as much as possible from the splenic pulp, and then hardened with corrosive sublimate or ammonium bichromate.

(2.) *S. Spleen of sheep, stained with hæmatoxylin. B. (Fig. 78.)*

Under the low power observe again the general arrangement of parts. Find in the spleen pulp the commencement of a vein—a venous radicle—cut longitudinally, and put on the high power. Find a portion of this radicle where the wall is complete as at (*b*) in the figure, and trace it backwards towards its origin between the cells of the pulp. Note how the wall first of all becomes interrupted by spaces occurring between the cells forming it, and finally ceases to exist as a definite wall at all. Note the cells forming the pulp anastomosing with each other by their flange-like processes. Now examine the cells in the meshes of the network and in the commencing venous radicle. Most of these are red blood corpuscles; some are leucocytes or lymph cells; some are large white blood corpuscles. Look, however, for larger cells than these—cells which may be regarded as peculiar to the situation. They are large round, oval, or polygonal cells, usually containing more than one nucleus, and, in addition, yellow pigment granules or unaltered blood corpuscles.

Examine the adenoid tissue forming a Malpighian corpuscle.

(3.) *Injected spleen of cat or rabbit. B.*

Under the low power note the very striking appearance of the section. The gelatine mass has infiltrated the entire spleen pulp, leaving the Malpighian bodies uninjected, except for the blood-vessels upon which they occur. We have thus scattered over the field round or oval unstained areas, containing an excentrically placed injected vessel. The rest of the specimen (with the exception of the capsule and trabeculæ) presents a deep red or blue appearance, according to the gelatine mass used. The contrast between the injected and non-injected parts is often very sharply defined.

In some cases, as when the injection has been more carefully made, the capillary network in the splenic bodies is, to some extent, revealed.

THE THYMUS GLAND.

The thymus gland is found in the anterior mediastinum of the chest. It arises as an outgrowth of the epithelium of the visceral clefts. It soon becomes, however, obviously *lymphoid* in its nature, and, no doubt, bears some relation to the formation of blood corpuscles. It commences to atrophy about the sixth year, and is ultimately replaced by fat and connective tissue. It is enveloped by a fibrous *capsule* sending in *septa*, which divide it into *lobules*. From these *septa* secondary divisions are given off, which partially subdivide the lobules into *follicles*.

A lobule (*Fig. 87*) may be taken as representative of the structure of the whole gland. It is pyramidal in shape, and possesses a cortex and a medulla. The cortex consists of a series of lymph nodules, each of which is in communication with the central stem or medulla. The nodules are separated from each other by fibrous septa sent in from the connective tissue enveloping the lobule. Both the cortex and medulla are composed of lymphoid tissue, but whereas in the cortex this is similar to that forming the lymph blocks in a lymphatic gland, in the medulla it is represented by a more open adenoid network, the meshes of which are larger, and contain fewer lymph cells. For this reason the medulla appears to be comparatively lightly stained.

In the medulla of a lobule there occur here and there small round bodies, varying greatly in size, which are termed *Hassall's corpuscles*. They consist of a few, or many, layers of concentrically arranged, flattened epithelial cells, surrounding a granular or corneous centre. The corpuscles do not stain well with hæmatoxylin, and are thus readily distinguished from the adenoid tissue which surrounds them.

If the section does not pass through the medulla of a lobule, but only through the cortex, it will appear to be made up of a number of isolated lymph nodules, with fibrous septa completely investing them.

(1.) *S. Thymus gland of child, stained with hæmatoxylin.*
B. (*Fig. 87.*)

With the low power, look over the specimen and note the general appearance. The piece of gland examined will, of course, consist of numerous lobules cut in various directions, united by fibrous tissue, carrying blood-vessels, nerves, and lymphatics.

FIG. 75.

COVER-GLASS PREPARATION OF HÆMAL GLAND, STAINED WITH METHYL-BLUE, UNDER $\frac{1}{16}$ -IN. IMMERSION LENS.

- a.*—Large faintly stained non-nucleated cells.
- b.*—Large cells, also faintly stained, but nucleated.
- c.*—Small cells, nucleated.
- d.*—Small cell, non-nucleated.
- e.*—Body being extruded from cell.
- f.*—Blood corpuscles.

FIG. 76.

COVER-GLASS PREPARATION OF HÆMAL GLAND, STAINED WITH FUCHSIN, UNDER $\frac{1}{16}$ -IN. IMMERSION LENS.

- a.*—Granular cells.
- b.*—Red blood corpuscles.

Fig. 75.

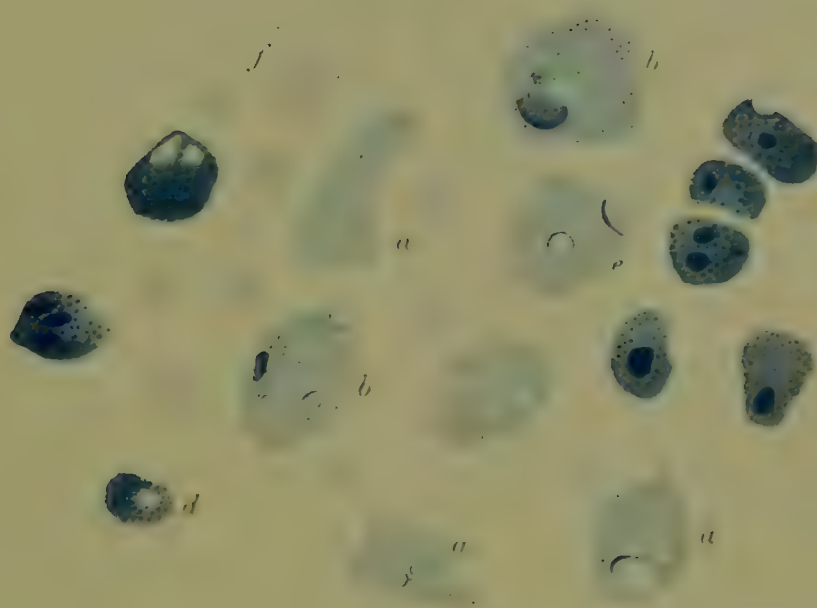
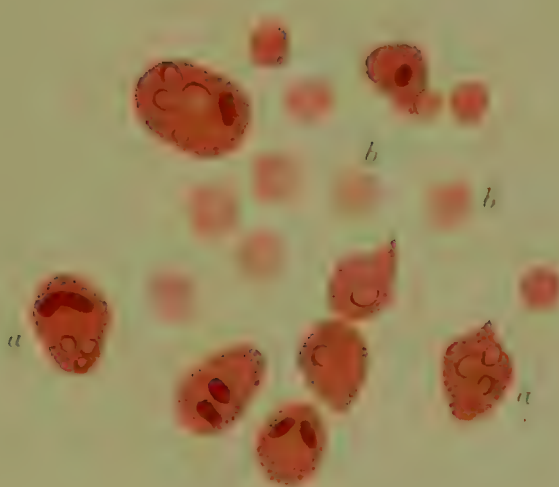


Fig. 76.



The arteries occurring in the interlobular septa of the thymus are often very typical of a small or medium-sized vessel. In a specimen stained with hæmatoxylin, the fibrous tissue appears pink, or yellowish pink, and the lymphoid tissue blue. Find a lobule somewhat pyramidal in shape, cut vertically through its medulla. Notice the arrangement of the nodules of lymphoid tissue, forming a deeply stained cortex, and the more lightly stained medulla or core. Note that each nodule is connected with the medulla on its inner aspect, but that, peripherally, each is separated from its neighbour by the secondary septa of connective tissue.

Recognise the faintly stained, circular Hassall's corpuscles in the medulla. Distinguish them from sections of blood-vessels. The latter contain blood which presents a distinct yellow or yellowish-green colour. Select one of these corpuscles, and put on the high power.

Under this power note the concentric arrangement of the elements of the body, and in the case of the smaller ones, the presence in the centre of one or two unflattened epithelial cells. The outlines of the individual sheathing cells are often not well seen.

THE TONSILS.

The tonsils, placed one on either side of the fauces, consist of aggregations of lymphoid tissue, forming a local collection beneath the stratified, squamous epithelium lining this part of the alimentary canal. The epithelium dips down between the lymph nodules to form crypts or pit-like recesses, into the bottom of which the ducts of mucous glands frequently open. The lower margin of the epithelium, where it lies in contact with a lymph nodule, tends to become "eroded," that is to say, the leucocytes penetrate between the epithelial cells and appear to work their way to the surface of the layer; the sharp line demarcating the epithelial from the lymphoid tissue being thus lost. The lymph nodules which frequently show *germ-centres*, are embedded in a more open adenoid form of connective tissue, and the whole is surrounded with a condensation or sheath of ordinary connective tissue.

(1.) *S. Tonsil (human), stained with borax-carmin.* B.
(Fig. 85.)

Under the low power observe the layer of epithelium (a), and

look for a recess similar to that shown in the figure. Find the lymph nodules (*b*) and mucous glands (*c*). Put on the high power and examine especially the 'erosion' of the epithelium, in parts, by the invasion of leucocytes from below.

Development of the Thymus Gland and the Tonsils. The original tubes of epithelial cells forming the commencement of the **Thymus**, divide dichotomously to form an organ possessing the usual lobular arrangement. This is rapidly followed or accompanied, by a proliferation of the gland cells, so that a system of solid tubes without a lumen results. The connective tissue immediately surrounding it becomes lymphoid in character, the whole structure being in turn enveloped in a condensation of ordinary connective tissue, representing the fibrous capsule of the fully developed organ. The continued proliferation of the gland cells results in the central cells of the tube being compressed and undergoing corneous degeneration, in much the same way as those in the "cell nests" of an epithelioma; and, co-incidentally, the tube becomes interrupted here and there owing to its removal by the growing lymphoid tissue by which it is surrounded. In this way isolated collections of epithelial cells (Hassall's corpuscles), entirely surrounded by lymphoid or adenoid tissue, result. After the complete removal of the original "glandular" structure, the lymphoid tissue itself becomes transformed to adipose tissue, the resulting fatty mass still retaining the characteristic lobular arrangement, which its immediate precursor, the lymphoid thymus, inherited from the original epithelial tube it enveloped and replaced. The **Tonsil** commences as an exvagination of the epithelium lining the fauces into the mesoblast outside it. This is succeeded by an infiltration of leucocytes immediately around it, the whole being, as in the case of the thymus, surrounded by a condensation of ordinary connective tissue. The adult organ results from an extension of the process, the original crypt ramifying, and additional ones appearing, while the leucocyte infiltration increases at the same time. The same accumulation of proliferated epithelial cells takes place in the crypts, as in the original gland tubes of the thymus; and were it not that the crypts remain open to the surface, and the contained degenerated cells can thus be thrown off, a condition analogous to that of "cell nests" would doubtless occur. In addition to the continued patency of the crypts, no doubt the invasion of the epithelium by leucocytes, and their escape into the cavity of the crypts, aids in the throwing out of the accumulated *débris* (Gulland).

HÆMAL GLANDS.

The above name may be given to certain "glands" which have been observed in the pig, horse, ox, and sheep, but which have not yet been found in man. There are two varieties of these structures: (1,) small, and very numerous; and (2,) a larger form, more nearly resembling a lymph gland, fewer in number, and less widely distributed than the first.

(1,) In the prevertebral fat of many herbivora are to be

found numerous small, usually round bodies, from a pin's head to a small pea in size, of a dark chocolate colour, and of a pulpy consistence. On account of their dark colour, they stand out very distinctly in contrast to the fat in which they are imbedded. They have hitherto been investigated in herbivora only, and are not to be seen in the human body, as it appears in the dissecting room. It is possible that more careful search in quite recent cadavera would not be without result.

These structures are invested with a thin capsule of connective tissue. Beneath the capsule is a sinus, not unlike the sub-capsular sinus of a lymph gland, in that its wall is formed of a layer of epithelioid cells, and it is traversed by a coarse adenoid reticulum. Here, however, the sinus contains not lymph, but blood. Within the sinus we have the main mass of the gland, which consists of lymphoid tissue with large spaces or sinuses in it containing blood. These spaces are in communication with each other at various points, and also with the peripheral sinus. The centrally placed spaces do not, however, contain an adenoid reticulum. The inner surface of the capsule, and the lymph follicular tissue throughout the gland, are covered with a layer of epithelial plates. The relations of the different parts are shown in *Fig. 73*.

The lymphoid tissue, which seems to resemble in every way that of a lymph gland, is variable in quantity and in arrangement in different glands from the same animal. It may be very small in amount, so as to form merely a network of cords separating the peripheral from the central sinuses, and these latter from each other; or it may, on the other hand, form the greater part of the organ, the central sinuses appearing as small, isolated lakes of blood in the general lymphoid mass. As to arrangement, it is very common, as in the figure, to find the greater part of the lymph follicular tissue towards the periphery, where it forms a more or less definite inner boundary for the peripheral sinus; on the other hand, the principal accumulation is sometimes towards the centre.

No account has yet been given as to the vascular and lymphatic supply of these glands—provisionally, we may suppose, with regard to vascularisation at least, that the afferent blood-vessels open into the sinuses, and the efferent emerge from them; that the sinuses, central and peripheral, may in fact be regarded as vascular dilatations.

FIG. 77.

S. CAT'S SPLEEN, STAINED WITH HÆMATOXYLIN \times 50.

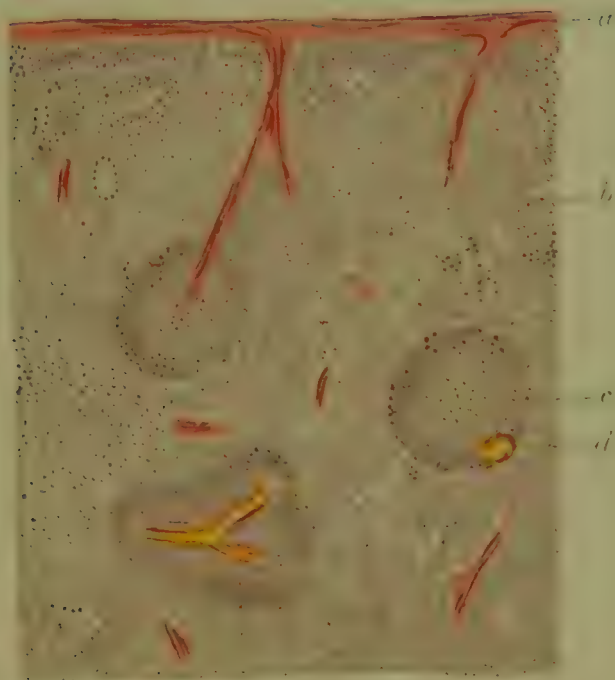
- a.*—Capsule, with trabeculæ passing into the substance of the spleen.
- b.*—Pulp tissue.
- c.*—Malpighian or splenic corpuscle.
- d.*—Artery in splenic corpuscle.

FIG. 78.

S. SHEEP'S SPLEEN (WASHED), SHOWING COMMENCEMENT OF VEINS IN PULP, STAINED WITH HÆMATOXYLIN \times 300.

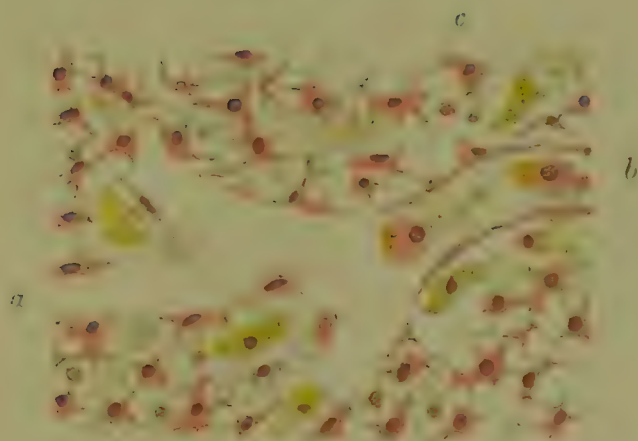
- a.*—Commencing venous channels.
- b.*—Venous radicle, with complete wall.
- c.*—Cells of spleen pulp.

Fig. 77.



77

Fig. 78.



78

With regard to function, there seems little reason to doubt that they are local centres for the production of blood corpuscles, both white and red, but the stages of formation have not yet been traced out.

If a cover-glass preparation of these glands be made by squeezing a little of the pulp upon a cover-glass, and treating it in the usual manner, the more intimate structure of the cells may be studied (*Figs. 75 and 76*). Many of them seem to be ordinary lymph cells with a single nucleus, and a small amount of peri-nuclear protoplasm; others seem to have increased in size, and stain less deeply with reagents. These frequently exhibit more than one nucleus, and their protoplasm contains a number of spherules of different sizes. These spherules may number from one to half a dozen or more, and may occur in any part of the cell. Occasionally they may be seen apparently being extruded from the corpuscles, as shown at (*e*) *Fig. 75*. They stain very lightly with methyl-blue, more distinctly with fuchsin. In the field between the white cells numerous red blood corpuscles (*f*) may be observed.

(2.) The second variety of hæmal gland is more nearly related to an ordinary lymph gland. Such glands are found in the prevertebral fat, but are especially localised in the region of the kidneys, where they may be found to the number of a dozen or more, clustered about the renal vessels. Like the smaller glands, they are soft and pulpy in consistence, and of a dark chocolate colour. On section they seem to have somewhat the same structure as a lymph gland, except that the medulla appears to be represented by a more or less compact mass of lymphoid tissue, rather than by the networks of trabeculæ and lymph cords; and the peripheral sinus is filled with blood. The lymphoid tissue in many parts of the organ possesses the characteristic of refusing to stain with the reagent, and under the high power the cells are seen to be increased in size. Cover-glass preparations are virtually identical with those obtained from the smaller variety.

Examine the following specimens:—

(1.) *S. Hæmal gland (small variety) of sheep, stained with borax-carminé. B. (Figs. 73 and 74)*

Under the low power, observe the parts of the gland as shown in *Fig. 73*: the capsule (*a*), the peripheral blood sinus (*b*), the central blood sinuses (*c*), and the lymphoid tissue (*d*); but it

must be remembered that these vary greatly in their form and disposition in different specimens. With the high power, study the peripheral sinus (*b*) *Fig. 74*, and the parts on either side of it. Note the network (*e*) by which it is traversed—a coarser one than that found in the sinus of a lymph gland; and that the meshes are here filled with blood. Amongst the mass of unstained, yellowish corpuscles filling the sinus, note the presence of other cells stained pink, nucleated, and round—the white corpuscles. Now examine the lymphoid tissue (*d*), observing the investing line of epithelium (*g*) with the nuclei of the cells seen in section here and there; the mass of lymph cells of which it is composed, with an occasional indication of a delicate adenoid reticulum between them; and the walls of the capillary blood-vessels (*h*). Observe that in some parts of the gland the cells of the lymph tissue have undergone some increase in size, and stain less deeply with the reagent.

(2.) *Cover-glass preparation of hæmal gland of sheep, stained with methyl-blue. B. (Fig. 75.)*

Use an oil immersion lens of as high a power as possible, and increase the light with a condenser. Observe the different appearances presented by the cells, noting especially the spherules in the larger, more lightly stained ones. The spherules themselves are still more lightly stained than the protoplasm of the cells in which they occur, and may at first sight be taken for vacuoles. Look carefully for an appearance of one being extruded, as shown at (*e*); and also find the red corpuscles (*f*) scattered over the field between the white.

(3.) *Cover-glass preparation of hæmal gland of sheep, stained with fuchsin. B. (Fig. 76.)*

Under the same power, observe similar cells in this preparation, but note that here the spherules in the larger cells are more distinctly stained by the reagent, as are also the red blood corpuscles which are free in the field.

THE THYROID GLAND.

The thyroid gland possesses two lobes, placed on either side of the trachea in the neck, and joined to each other by an isthmus crossing in front of it. The gland possesses a fibrous capsule, containing blood-vessels, nerves, and lymphatics. The capsule sends septa into the interior of the organ, which surround

and separate the ultimate alveoli from each other. These alveoli are usually round or oval isolated spaces, lined by a layer of cubical or low columnar epithelium, and filled with a mass of mucoid or colloid material. The alveoli have no duct communicating with them, though in development the gland arises as an exvagination from the alimentary canal. The original duct, however, rapidly disappears.

S. Thyroid gland of child, stained with hæmatoxylin.
B. (Fig. 86.)

Under the low power, observe the capsule of the gland, and trace it into the interior in the form of septa or trabeculæ. Note the rounded masses of colloid material of which the gland seems to be mainly composed, and, lining the spaces in which they lie, the layer of cubical epithelial cells, which under this power appears to be little more than a line of nuclei stained deeply with the hæmatoxylin. Very frequently the colloid masses are seen retracted from the wall of the alveoli, in some cases carrying the layer of cubical epithelium with them. Under the high power, examine especially the wall of the alveolus, and identify the layer of cubical epithelium. When the colloid mass is retracted, its outline is often scalloped in a peculiar manner, due, perhaps, to its being more adherent to the epithelium at the junctions between the cells, than over their surface. Sometimes fine threads may be seen connecting the retracted colloid material with the junctions. Notice that, in some cases, the knife has passed not through the centre of an alveolus, but through its margin, so that a surface view, as it were, of the epithelial lining is obtained. Note that the colloid material may frequently also be found in the lymph channels in the inter-alveolar septa.

THE PITUITARY BODY.

The posterior lobe, developed as an outgrowth from the epithelium of the mouth, consists of a number of round, or oval or elongated alveoli, somewhat similar to those of the thyroid gland, and, like them, separated from each other by septa of connective tissue. The alveoli do not, however, for the most part, possess colloid contents, but are usually solid masses of cells columnar or polyhedral in shape.

The anterior lobe is a part of the central nervous system, and

FIG 79.

S. SUPRARENAL GLAND OF DOG, STAINED WITH HÆMATOXYLIN $\times 250$.

A.—Cortex.

B.—Medulla.

a.—Zona glomerulosa.

b.— „ fasciculata.

c.— „ reticularis.

d.—Capsule.

e.—Capillary blood-vessel.

f.—Alveoli of medulla.

g.—Vein in medulla.

FIG. 80.

S. SUPRARENAL GLAND OF DOG (MEDULLA), STAINED WITH HÆMATOXYLIN $\times 350$.

a.—Alveolus cut transversely.

b.— „ „ longitudinally.

c.—Capillary blood-vessel between the alveoli.

Fig 79.

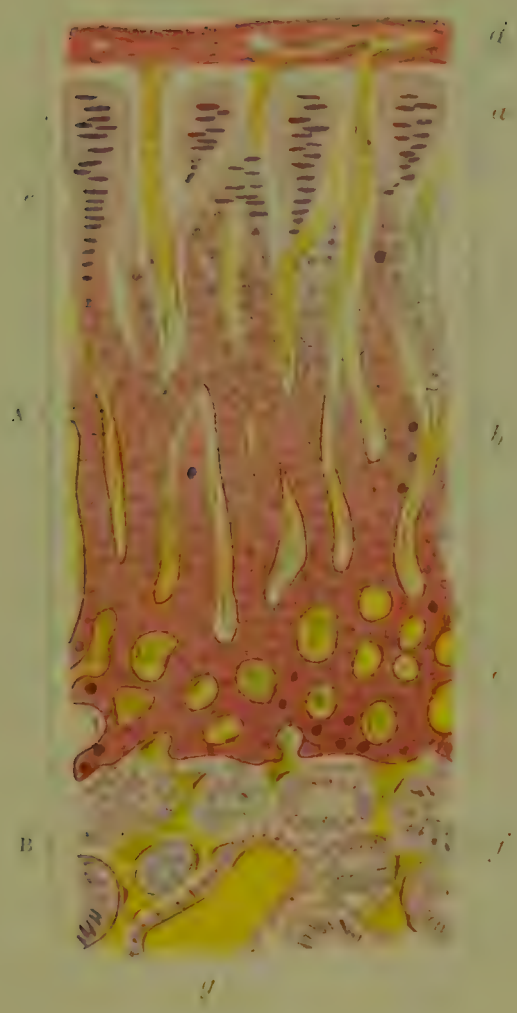


Fig. 80.



is a continuation of the infundibulum. Examine a section of the posterior lobe, and observe the closed alveoli usually completely filled with cubical, columnar, or polyhedral cells, and exhibiting no lumen.

THE SUPRARENAL CAPSULES.

The structure of these organs varies considerably in different species. The supra-renal of the dog may be considered as fairly typical of the mammalian gland, but some others will also be described.

The Supra-renal gland of the dog consists of an outer cortical, and a central medullary portion. The *medulla* is everywhere enveloped by the *cortex*, except at the hilum, where it reaches the surface. The organ is invested with a fibrous capsule, which sends inwards delicate septa containing blood-vessels; and these pass from the periphery to the centre of the medulla, which they leave by the large, efferent vein at the hilum. The main part of the cortex is composed of a system of radially arranged columns of cells, not unlike those found in a lobule of the liver, which anastomose with each other. The cells forming them are hexagonal and polygonal in shape, and apparently belong to the variety of epithelium termed "glandular." The columns terminate immediately beneath the capsule in blind extremities, and neither in these nor in the columns themselves is any lumen to be made out. If they are traced towards the medulla they are again found to end abruptly. The columnar arrangement is most obvious in the central, broader portion of the cortex, which is hence called the *zona fasciculata*; beneath the capsule the blind extremities of the columns (usually broader than the columns themselves) are frequently cut transversely, and the appearance resulting has given to this region the name of *zona glomerulosa*; while at the inner margin of the cortex, the columns lose their fasciculate appearance, and, anastomosing more freely with each other, form a network, the *zona reticularis*.

In all parts of the cortex are to be found capillary blood-vessels, supported with delicate connective tissue, lying between the columns of cells, and forming an alternate network with them. The cortex is somewhat sharply marked off from the medulla, by a narrow layer of ordinary connective tissue continuous with the general supporting framework of the

gland. The medulla seems to consist almost entirely of closed gland alveoli which possess the following characters. They are usually rounded in shape or polygonal from the pressure, as it were, of neighbouring alveoli, and lined by tall columnar cells, the nuclei of which are placed at the central end, around a very small lumen. Between the alveoli is a network of capillary blood-vessels, dilated here and there to form large spaces or venous channels. These seem to have, like the capillaries, no wall save an epithelial one; or if there is a connective tissue element outside this, it is a very slight one. In some animals, such as the sheep, where there is a single, large tubular space in the centre of the medulla, serving as the commencement of the efferent vein, a fibrous layer outside the epithelium is very marked; and in man the venous radicles possess also a layer of non-striped muscle fibres. The alveoli of the medulla are apparently closed, and there is no evidence of an excretory duct in connection with them. This part of the gland is freely supplied with sympathetic nerve fibres, which are said to be accompanied by groups of nerve cells; but the latter are not always to be made out.

The **Suprarenal gland of the sheep** is a small round, or, perhaps, reniform organ in close contiguity to the anterior end of the kidney, but not, as in the human subject, forming a cap fitted upon it. On section it presents a broad, lighter coloured cortex, and a central, more deeply coloured medulla, which reaches the surface at the hilum. Sections may advantageously be stained with saffranin, which yields very accurate definition of the cell elements.

The cortex is divisible like that of the dog's gland, into three zones, the innermost of which is so similar to the middle, of which it forms a part, that it scarcely deserves a separate name. The *zona glomerulosa* is equal in breadth to about one-fifth of the whole cortex, and consists of a number of alveoli, which are represented in *Fig. 83*. They are round, or oblong, or convoluted in outline; lined by polygonal, granular looking, nucleated cells, not unlike those of the convoluted tubes of the kidney; which surround a central lumen into which they may so far project as to meet in the middle of it. Sometimes, as shown in the figure, there is a distinct space between the individual cells, which may, however, be due to the process of hardening. Between the alveoli are prolongations of delicate connective tissue from the capsule

bearing capillary blood-vessels. There appear to be two or three layers of these alveoli before the *zona fasciculata* is reached. The latter consists of a network of polygonal glandular cells, resembling very closely the network of cells found in a lobule of the liver. A distinctly striated appearance to the naked eye is given to this portion of the cortex, owing to the arrangement of the network, as in the dog, in the form of radial columns of cells with lateral anastomosing cords. Towards the innermost part of the cortex, that to which the term *zona reticularis* is given, the arrangement in radial columns is lost, and the network is more an irregular one. The columns, as stated, resemble very closely those of the liver. The cells in shape are almost indistinguishable from liver cells. They are polygonal, often pentagonal, with clearly defined outlines. Each cell possesses a nucleus with a nucleolus. Between the cords of cells we have an alternate system of blood capillaries continuous with the capillary network between the alveoli of the *zona glomerulosa*. The delicate connective tissue carried in from the capsule seems to be almost entirely deficient, and the capillary walls are closely applied to the cell columns. Indeed, this is frequently the case in the *zona glomerulosa*, as shown in *Fig. 83*.

At the point where the *zona glomerulosa* and *fasciculata* meet, the alveoli of the one are directly continuous with the cords of the other; that is to say, the alveoli in the inner portion of the *zona glomerulosa* join on to the cords of which they appear to be the expanded extremities. The appearances, in fact, would lead one to suppose that the alveoli are sections of the convoluted expanded extremities of the columns of cells forming the *zona fasciculata*.

When the junction between the cortex and medulla is approached (*Fig. 84*), the cortical gland tubes lose, as before said, their columnar, radial arrangement, and form a more or less irregular network, which becomes less open, that is to say, with smaller intervening capillary spaces as the inner edge of the cortex is reached. The cortex appears more deeply stained with the reagent than the medulla, and the line of demarcation is quite distinct; there being apparently no connection between them, except through the continuity of the capillary blood-vessels. The comparatively lightly stained medulla is seen to consist of a number of more or less tortuous convoluted tubes, broader very considerably than the cords of cells in the cortex, and separated

FIG. 81.

L.S. SUPRARENAL GLAND OF CAT, STAINED WITH METHYL-BLUE, UNDER
ZEISS A₂ LENS.

- a.*—Capsule.
- b.*—Zona glomerulosa of cortex.
- c.*— „ fasciculata „
- d.*—Medulla.
- e.*—Hilum.
- f.*—Zone of enlarged cells in zona fasciculata.

FIG. 82.

S. SUPRARENAL GLAND OF CAT, STAINED WITH HÆMATOXYLIN × 350.

- a.*—Unaltered cells of zona fasciculata (outer part).
- b.*—Cells unstained by the reagent, but coloured naturally a golden yellow.
- c.*—Enlarged unstained cells, showing fat globules and intra-cellular network.
- d.*—Fat globules, unstained.

Fig. 81.

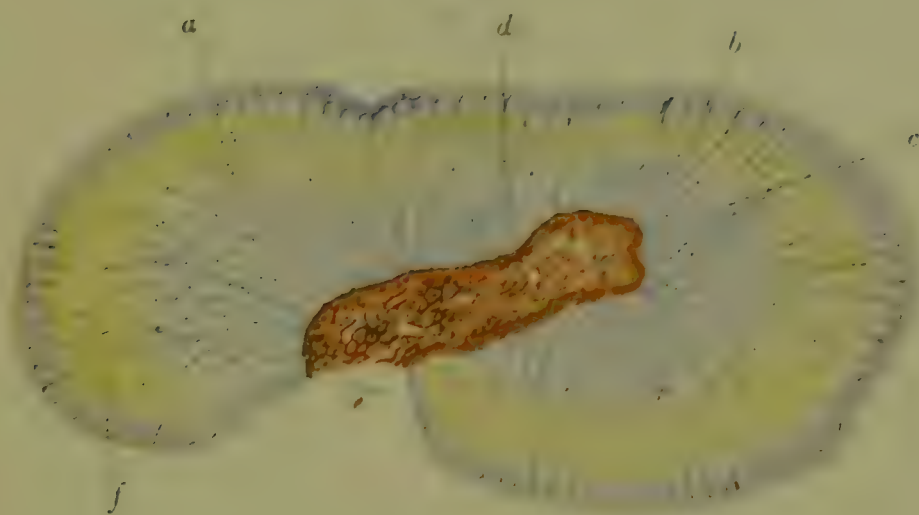


Fig. 82.



from each other by a vascular network continuous with that found in the cortex. The spaces between the gland tubes or alveoli, which constitute the parenchyma of this part, are not tubular, as blood capillaries generally are, but quite irregular, their shape depending on the outline of the adjacent tubes, between which they lie. Their walls seem to be formed like those of the capillaries of the cortex of a layer of squamous cells closely applied to the outside of the glandular alveoli.

At first sight these alveoli have no lumen, and on further examination this is confirmed throughout the greater part of the medulla; but here and there sections of alveoli may be found in which the lumen is quite distinct. They are lined with very tall columnar cells, which usually meet those opposite them in the centre of the tube, and which have their nuclei not in their basal portion, but close to their central ends, so that in many cases it looks at first sight as if the lumen of the alveolus was occupied with nuclei.

In the centre of the medulla is the large efferent venous channel lined with squamous epithelium, and surrounded with a layer of fibrous tissue. The channel extends through the medulla and terminates in the efferent vein at the hilum. The capillary vessels lying between the alveoli are in connection with this central channel. Here and there the capillary system widens out into larger, irregular spaces between the alveoli. Sections of non-medullated nerve bundles occur here and there, supported with delicate connective tissue, in which are also to be seen sections of small blood-vessels.

Before leaving the description of the histology of the sheep's suprarenal, a certain peculiarity in the arrangement of the columns of cells in the cortex must be referred to. When a section passing through the centre of the gland (and consequently cutting these columns in their long axis) is examined, it is seen that the columns are arranged in alternating bundles; the columns in one bundle being in close proximity to each other, and in those on either side of it more widely separated. This arrangement is exceedingly regular and striking, and presents somewhat the appearance of the pyramids of Ferrein in the kidney, with the intervening interpyramidal cortex. That this is no accidental arrangement is well brought out in a section of the gland cut through the zona fasciculata, transversely to the axis of the columns, one which does not pass through the

medulla. In the central part of such a section the alternate arrangement of these bundles is very diagrammatically seen; those in which the tubules were near to each other, presenting the appearance of a network with very small meshes; those in which they were further apart showing a network with larger meshes.

The **Suprarenal gland of the cat** (*Fig. 81*) is, in proportion to the size of the animal, rather a large one. It is reniform in shape, and placed in close relation to the kidney. On section it presents the usual appearance of a cortical and medullary division, the latter deeply coloured, and extending to the surface of the organ at the hilum. The suprarenal of the cat is remarkable for the pigmentary and other changes which it is liable to undergo, but in many cases it is found to be entirely unpigmented, and it may be well to describe it in this condition first. It is surrounded by a capsule, which sends in delicate connective tissue trabeculæ, bearing blood capillaries as in the sheep. The parenchyma of the cortex is, however, a little different in its character. The *zona glomerulosa* here consists of one layer only of alveoli, which have a closed extremity immediately beneath the capsule, and an open one, if it may so be called, towards the columns of the *zona fasciculata*, which they join. The *zona glomerulosa* is thus much narrower than in the sheep. The trabeculæ passing in from the capsule lie on either side of each alveolus, and thus help to enclose it in a kind of three-sided box, the fourth side, where the alveoli join the columns of the *zona fasciculata*, being, of course, wanting. The *zona fasciculata* is composed of columns of cells more closely arranged than in the sheep, so that the capillaries between them do not always show us open spaces, but often merely as lines indicated by their walls. The columns anastomose with each other laterally, and at the inner portion of the cortex lose their columnar, radial arrangement as before, and form an irregular network, the *zona reticularis*.

The cells, both in the alveoli of the *zona glomerulosa* and in the columns and in the *zona reticularis*, are essentially similar to those in the sheep. The medulla resembles that of the sheep's suprarenal very closely. It does not appear, however, to possess a large central channel, but the blood appears to be collected into a number of large irregular spaces lying between the alveoli—spaces precisely similar to those previously described in

the sheep's medulla. As before, these spaces do not present the outline of an ordinary vein; they are not rounded, but their sides are formed of a series of convexities inwards, the convexities of the alveoli among which they are placed. These spaces, as in the sheep, communicate with each other by narrower channels, and open at the hilum into the efferent vessel. They are lined by a layer of endothelial cells. Here and there in the medulla, between the alveoli, and supported by a small amount of delicate connective tissue, small arteries and veins are to be seen, and also a few sections of bundles of non-medullated nerve fibres, but, apparently, no nerve cells.

If we now examine a specimen in which pigmentation has commenced, certain well-marked changes are to be observed, both in the cortex and the medulla. In the cortex, the cells of the columns in the outer third of the zona fasciculata increase in size to sometimes twice their original bulk. Their protoplasm ceases to stain with the reagent, and exhibits a very distinct network (*Fig. 82*). These enlarged cells are of a pale straw colour, and the lumen of the capillaries between the columns is often quite obliterated.

Under the low power such an appearance as is represented in *Fig. 81* is produced, viz., that of a yellowish band lying immediately within the zona glomerulosa. In a more advanced stage of pigmentation this band becomes broader as the condition extends towards the medulla. It does not usually reach the medulla, though it may involve nearly the whole breadth of the zona fasciculata. *Fig. 82* shows the change the cells undergo when the condition is well advanced. Here and there they exhibit large round fat globules in their interior. As will be seen in the description of the medulla, somewhat similar bodies also occur in the cells of the alveoli there. Amongst these cortical cells it is to be observed, too, how some of the cells have not enlarged, but remain amongst their enlarged neighbours in their original condition, and thus present a marked difference in colour. Others, again, while not enlarging, have become pigmented almost a golden yellow. The capillaries are shown in the figure merely as lines between the swollen tubules.

In the case of the medulla, when the pigmentation is at all advanced, the change is so great that the alveolar arrangement is, at first sight, entirely lost, and from the examination of such a specimen alone it would be difficult to determine it. The cells

FIG. 83.

S. SUPRARENAL GLAND OF SHEEP, STAINED WITH SAFFRANIN (SHOWING ALVEOLI OF ZONA GLOMERULOSA) $\times 300$.

a.—Alveolus.

b.—Capillary wall.

FIG. 84.

S. SUPRARENAL GLAND OF SHEEP (SHOWING JUNCTION OF CORTEX AND MEDULLA), STAINED WITH SAFFRANIN $\times 200$.

a.—Cortex.

b.—Medulla.

c.—Capillary blood-vessel.

Fig. 83.

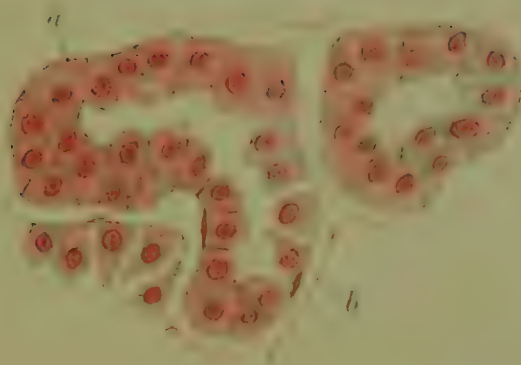
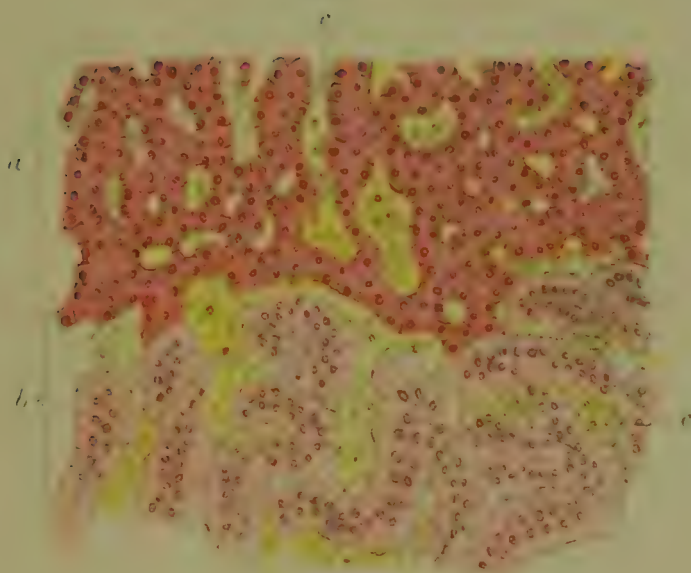


Fig. 84.



lining the alveoli become very deeply pigmented, the colour being of a rich brown, almost a vandyke, and lose their regular shape. It is no longer possible to distinguish them as clearly defined columnar cells, arranged round a central point. (This pigmentation is said to be due to the action of bichromate of potassium used in preparing the specimen. But the alteration in the shape of the cells and the appearance in them of the bodies about to be mentioned, are probably indications of functional changes, as are also those already described as taking place in the cortex.) At the same time, a round body, which does not stain with the usual reagents, and is apparently a fat globule, appears in many of them, and forcing the protoplasm together with the nucleus to the periphery, gives to it somewhat the appearance of a signet ring, the signet being represented by the nucleus with part of the protoplasm around it, and the hoop of the ring by the remainder of the protoplasm and the cell wall, or by the latter alone. But this change is not the only one. In many of the pigmented cells small, bright yellow spherules make their appearance. Sometimes they are about the size of a red blood corpuscle; more usually, however, they are somewhat larger. There may be half-a-dozen of these spherules in one cell, or there may be only one. They occur in the perinuclear protoplasm, and the cell may or may not show one of the large unstained bodies, at the same time, in another part. Usually, they do not, as there is not room left for the spherules in most of the cells which have assumed a signet ring appearance.

Before leaving the consideration of the suprarenal body of the cat, it may be mentioned that the change in the cortex and medulla advance side by side. The zona glomerulosa and zona reticularis appear to remain up to a certain point unaltered, but when the pigmentation of the zona fasciculata and medulla is far advanced, the cells of the zona reticularis are apt to become pigmented in their turn, and, sometimes, to show vacuolation. They do not, however, enlarge and become light in colour, like the cells of the peripheral part of the zona fasciculata.

The Suprarenal gland of man fits the upper part of the *kidney* as a kind of cap. The sections, when cut in the principal plane, are tri-radiate in shape. The zona fasciculata is a little remarkable from the extreme definiteness of the columns of cells, which do not communicate laterally with each other to the same extent as in most other mammals. Towards the zona reticularis

the communication between the cords of cells is peculiarly free, and in a section cut at right angles to the axis of the columns ending it, gives the appearance of a network of great regularity.

The medulla does not differ markedly from that of the sheep, and other mammals. It is easy to distinguish its alveolar nature. There is a very distinct central vein, possessing a considerable amount of non-striped muscle in its wall. It is rather difficult to understand the purpose of so much muscular tissue, seeing that it is quite absent from the central vessel in the medulla of some animals. The cells of the alveoli of the medulla show, here and there, the vacuolation described in that of the cat, and undergo a certain amount of pigmentation, the degree of which varies in different specimens, but no such marked change as that noted in the medulla of the cat is to be observed. The line of demarcation between the cortex and medulla is quite distinct.

In the suprarenals of some **amphibians** the cortex and the medulla are entirely separated from each other; in the **common fowl** they are not only combined in one organ, but intermingled.

The student is recommended to examine sections of the suprarenal glands of different species. As the steps are the same in each case however, only one will be considered here in detail.

(I.) *Radial section of suprarenal capsule of dog, stained with hæmatoxylin.* B. (Figs. 79 and 80.)

With the low power observe the capsule (*d*) surrounding the organ, and beneath it the broad cortex (A), sharply defined from the central medullary portion (B), which, however, reaches the surface at the hilum. Distinguish the three described layers of the cortex; the zona glomerulosa (*a*) at the periphery, immediately beneath the capsule, presenting the appearance figured in the drawing; the zona fasciculata (*b*), composed of radially arranged columns of cells, forming the main body of the cortex; and the zona reticularis (*c*), forming the innermost portion. The latter is not so easily distinguished as a special region of the cortex as the zona glomerulosa is, but with a little care it may be seen that here the cells seem to be no longer arranged in radial columns, but rather in the form of a network. Under this power the medulla appears to be made up of alveoli of epithelial cells, the character and arrangement of which is not very distinguishable; between the alveoli are seen numerous spaces, or venous sinuses, and in the neighbourhood of the hilum (if the section has

passed through it) may be seen a portion of the large efferent vein.

Find the capsule again, with the zona glomerulosa beneath it, and put on the high power. Note the structure of the capsule ; ordinary connective tissue containing, here and there, blood-vessels and lymphatic channels, and sending down from its deeper surface delicate trabeculæ, containing capillary vessels between the radial columns of cells. Now examine more particularly the zona glomerulosa (*a*). It consists of the blind extremities of epithelial tubes, cut usually longitudinally. Some of these blind extremities become turned upon themselves immediately beneath the capsule, much as if they were too long, and had in consequence been folded round. These blind extremities are broader than the columns with which they are connected, and the protoplasm of the cells lining them is less granular, and stains less deeply with reagents. In shape the cells lining them appear tall and columnar, and possess well-marked oval or elongated nuclei, which are placed in their long axis, and towards their inner extremities. In the suprarenal of the dog, horse, and some other animals, there seems to be no lumen within these blind extremities, but a lumen is very obvious in that of the sheep.

Now pass inwards through the cortex and examine the radial columns of cells, with capillaries between them. Note the polygonal shape of the nucleated, glandular, epithelial cells, and that, as in the liver, these are frequently arranged one in a row, but that sometimes there are two, or even more, abreast in a column. Observe that the fasciculi of cells are not isolated from each other, but communicate by branches passing off from them at an acute angle. Further inwards, constituting the inner boundary of the cortex, examine the zona reticularis, forming a network of cells almost indistinguishable from that of the mammalian liver, with blood capillaries between the strands. Now examine the medulla for an appearance shown in *Fig. 80*. Note the alveoli (*a*) cut transversely, and others cut longitudinally, as at (*b*), from which it appears that they are somewhat elongated, or tubular, in shape. (This is seen also in the figure of sheep's suprarenal *84 b*.) They usually appear quite solid, but sometimes a small lumen is to be seen. The cells lining them are tall and columnar in shape, and their oval nuclei are placed at their inner extremities, so as to abut on the lumen or central point.

FIG. 85.

S. TONSIL (HUMAN), STAINED WITH BORAX-CARMINE $\times 20$.

- a.*—Epithelium.
- b.*—Lymphoid nodule.
- c.*—Mucous glands.
- d.*—Blood-vessels.

FIG. 86.

S. THYROID (HUMAN), STAINED WITH HÆMATOXYLIN $\times 250$.

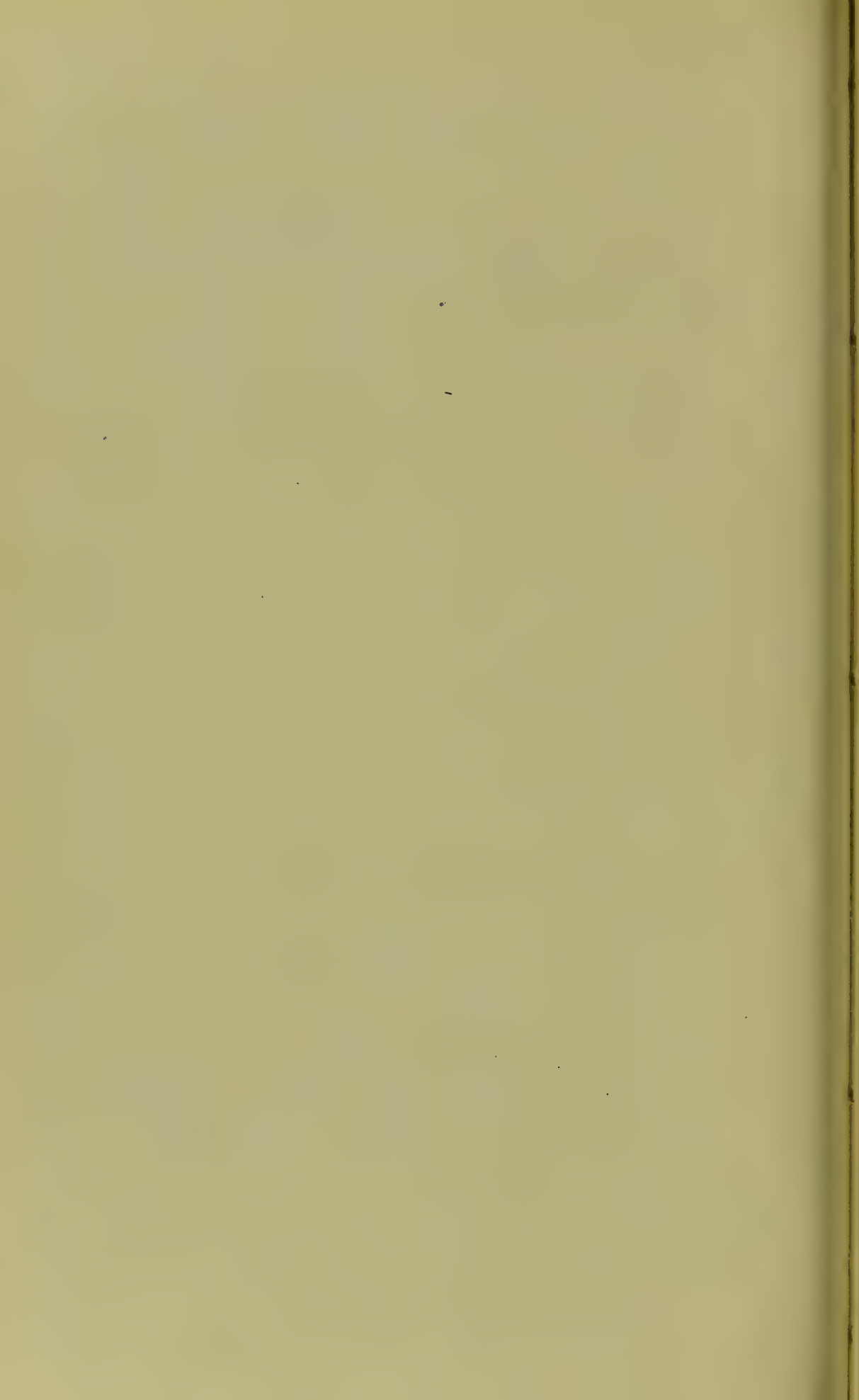
- a.*—Capsule.
- b.*—Fibrous septa.
- c.*—Alveolus lined with,
- d.*—Cubical cells.
- e.*—Homogeneous contents of alveoli.
- f.*—Surface view of alveolus.
- g.*—Artery.
- h.*—Irregular outline of retracted contents of alveolus.

Fig. 85.



Fig. 86.





Between these alveoli, which are often also polygonal in outline with rounded corners, observe the capillary network (*c*). Look for one of the sinuses. These appear to be merely spaces lined with epithelium, between the alveoli, and are thus irregular in shape.

Functions of the thyroid, pituitary body, and suprarenal capsules.—These organs, possessing no ducts, give rise to no *external* secretion. But their effect upon metabolism is very marked, and this is due to the fact that they produce what is termed an *internal* secretion : *i.e.*, certain substances are taken up by them from the blood, and, after elaboration, are returned to it in another form. A gland, such as the liver, has both an internal secretion—sugar, and an external one—bile ; the former of which passes into the blood, while the latter is discharged through the duct of the gland into the duodenum.

Our knowledge of the exact functions of the three ductless glands mentioned above is not great, and is derived from observations upon the result of their removal, by disease or otherwise, and the injection of fluid extracts of their substance into the blood stream. For a full account of the results of experimental work, the student is referred to the literature on the subject, but it may be shortly stated here that in Addison's disease, in which the suprarenals are affected, the patient is affected with muscular weakness, vascular alterations, and bronzing of the skin ; if extract of the medulla of these glands be injected into the veins of animals, the arteries are greatly contracted, and the blood pressure rises (Oliver and Schäfer). When the thyroid gland is hypertrophied, the condition of exophthalmic goitre results, in which increased excitability of the vascular system is one of the pronounced symptoms ; when it is removed the condition of myxœdema results, in which muscular weakness and tremors are associated with a dry and œdematous skin. This condition of myxœdema may be cured, or at least removed temporarily, by the injection of thyroid extract subcutaneously, or by its introduction into the stomach by the mouth.

APPENDIX TO CHAPTER IX.

METHODS OF PREPARATION.

1. Spleen of cat.—Harden in Müller and spirit, cut in gum transversely to long axis of organ, stain in picro-carmine, or hæmatoxylin, and mount in Farrant or balsam; or stain in bulk in borax-carmine, and cut in paraffin. Sections cut in gum may also be stained by the Erhlich-Biondi method. For a general view of the organ, sections cut in gum, stained in hæmatoxylin, and mounted in balsam, are very satisfactory. If it be desired to show the non-striped muscle, they may be additionally treated with eosin.

2. Spleen of sheep.—Use the spleen of a sheep for demonstrating the spaces of the pulp and the venous radicles. Wash out the blood from the organ by injecting gently warm saline solution, and then inject the fluid in which it is to be hardened (bichromate of potassium 2 per cent. solution, or Müller's fluid); cut in pieces, and place them in a large quantity of the same. After hardening, which may be completed in spirit, stain in bulk in borax-carmine, and cut in paraffin. Or after washing out with salt solution, place small pieces in 1 per cent. osmic acid for twenty-four hours, and then in spirit for a similar period; stain in bulk in borax-carmine, and cut in paraffin. Gum is useless for thin sections of "washed" spleen, as they invariably fall to pieces in the water.

3. Injected spleen.—Use the spleen of a cat or rabbit, which has been successfully injected from the aorta with blue or red gelatine mass. Harden in Müller and spirit, cut in gum, and mount in balsam.

4. Thymus of child.—The child should be less than five years old. Harden in Müller and spirit, cut in gum, stain in hæmatoxylin, and mount in balsam; or in picro-carmine, and mount in Farrant; or in alum-carmine, and mount in balsam.

5. Tonsil (human).—The organ should be got as fresh as possible, as it rapidly undergoes *post-mortem* change. Harden in Müller and spirit, cut in gum, stain and mount as 4.

6. Tonsil (of cat).—The cat's tonsil has the advantage of being usually in better condition. Harden (1,) in picric acid; (2,) in Flemming's fluid; or (3,) in Müller and spirit. Cut in gum, stain sections in hæmatoxylin, alum-carmine, or picro-carmine, and mount in Farrant or balsam. Or stain in bulk in borax-carmine, and cut in paraffin. Or, especially when hardened in Flemming's fluid, cut in paraffin, stain in saffranin, and mount in balsam.

7. Hæmal glands of sheep.—Get a sheep's kidney, with as much as possible of the fat in which it is placed. The parts should be obtained immediately

after death, before the fat has had time to set. Look for the first variety of hæmal glands as small blue, or chocolate coloured points, about the size of a pin's head, or rather larger, amongst the fat lobules. Also for the larger, second variety, about the size of small lymph glands, surrounding the renal vessels. Care must be taken in removing them, especially in the case of the first, as they are very readily crushed. Use one, either large or small, for *cover-glass preparations*. Make a section through it with a sharp razor, and rub the cut surface on two or three cover-glasses successively. These, when dry, may each be stained in a special manner (with methyl-blue, fuchsin (magenta), etc.), and mounted in balsam. For *sections*, place the glands in excess of Müller and spirit, or picric acid. On the second or third day divide the larger ones into two pieces; the smaller ones can be left as they are. When hardened, stain in bulk in borax-carminé, and cut in paraffin.

8. Thyroid gland (human).—Harden in Müller and spirit, cut in gum, stain in picro-carminé, or hæmatoxylin, and mount in Farrant or balsam; or stain in bulk in borax-carminé, and cut in paraffin.

9. Supra-renal capsule (mammalian).—Obtain a human suprarenal, and also that of dog, cat, and sheep. Incise, harden in Müller and spirit, cut in gum, stain in hæmatoxylin, and mount in balsam. Other stains may also be used, picro-carminé (Farrant), methyl-blue (balsam), or saffranin (balsam). Or stain in bulk in borax-carminé, and cut in paraffin.

10. Suprarenal of bird.—Dissect out the suprarenal from the anterior end of the kidney of the common fowl. It is well to remove the entire chest wall previously. Harden in Müller's fluid and spirit, stain in bulk in borax-carminé, and cut in paraffin. It may also be cut in gum, and stained and mounted as usual, but is apt to fall to pieces if the sections are sufficiently thin.

CHAPTER. X.

THE RESPIRATORY SYSTEM.

THE TRACHEA, BRONCHI, AND LUNGS.

THE general principle of construction of the lung is readily studied in that of the newt or frog. The newt's lung represents an exceedingly simple type ; that of the frog, a step between it and the more complicated structure found in mammals. But whether simple or complicated, the principle is the same throughout.

The lung commences as a diverticulum from the alimentary canal, and morphologically resembles a secreting gland. The newt's lung may be regarded as corresponding to a simple undivided gland, such as its own cutaneous ones, the trachea representing the duct, and the sac the alveolus of the gland. In the mammalian lung, on the other hand, the trachea divides repeatedly in the same way as the duct of a compound gland, the resulting subdivisions terminating in an extended system of alveoli.

As in a secreting gland, the alveoli are surrounded by a capillary network, supported by the connective tissue in which it lies. But whereas, in secreting glands, we have to deal with an epithelium which withdraws the materials necessary for its secretion from the lymph with which it is bathed ; in the case of the lung we are concerned with an epithelium which will permit of an interchange of oxygen and carbonic acid, between the air on the one hand, and the blood on the other. The process is probably one of diffusion of gases through a thin membrane.

The lung of the *newt* may be regarded as an elongated sac, with a tube, the trachea, leading into it. The sac is readily distensible by air, forced into it through the trachea, and as readily contracts again when the pressure is removed, owing to the

elastic character of its wall. As in secreting glands, we have here to deal with epithelium lining the cavity, and with connective tissue outside it, supporting blood-vessels. In this case the epithelium is of the simple squamous variety, the connective tissue outside it being in the form of a network of elastic fibres. The blood-vessels form a capillary network immediately below the epithelium, to some extent between it and the elastic tissue, and to some extent buried in the latter. Thus it will be seen that, between the blood in the vessel and the air in the sac, there is the thickness of two layers of squamous epithelium; on the one hand, the epithelium of the capillary wall, and on the other, that lining the cavity of the sac. At the opening of the trachea the epithelium changes in character, becoming columnar and ciliated, in which form it is continued along the tube till it becomes continuous with that of the pharynx. In the wall of the sac non-striped muscle cells are to be found here and there, running in the outer part of the elastic tissue in a transverse or circular direction; and a circular layer of non-striped muscle is also found in the trachea.

However complicated in detail of structure the lung may become, as we pass from this simple typical form to that found in the mammalia, the general principle of its structure is never departed from. We have always to deal with an elastic sac, consisting of a layer of squamous epithelium, resting on a basis of elastic fibres, supporting a network of capillary blood-vessels.

The *frog's* lung may be regarded as an intermediate form. It largely resembles the newt's, but is broken up into compartments by the projection inwards of septa of the subendothelial tissue, bearing with them the capillary network, covered on either side with the layer of squamous epithelium. In the upper part of the lung especially, we have these primary septa giving rise to secondary, and these again to tertiary septa; but this subdivision gradually ceases to take place as we pass towards the lower end of the sac. Thus, whereas the newt's lung is a single sac, the frog's is split up into a number of intercommunicating compartments, by the infolding of the wall at many points; such an infolding consisting of a basis of elastic fibres, supporting a capillary network, covered on either surface with a layer of simple squamous epithelium. As in the newt, the squamous epithelium of the lung becomes columnar in shape, and ciliated at the point where it passes into the trachea.

FIG. 87.

SECTION OF LOBULE OF THYMUS GLAND OF YOUNG CHILD, STAINED
WITH HÆMATOXYLIN $\times 40$.

- a.*—Fibrous investment of lobule.
- b.*—Cortex.
- c.*—Medulla.
- d.*—Hassall's corpuscles.
- e.*—Blood-vessels.

FIG. 88.

T.S. HUMAN TRACHEA, STAINED WITH HÆMATOXYLIN $\times 50$.

- a.*—Ciliated epithelium.
- b.*—Adenoid tissue.
- c.*—Elastic tissue.
- d.*—Glands.
- e.*—Blood-vessel.
- f.*—Lymphatic.
- g.*—Perichondrium.
- h.*—Cartilage.

Fig. 87.



Fig. 88.



The lung of a *mammal*, such as the cat, corresponds with a compound racemose gland, such as the sublingual or submaxillary. We have the trachea corresponding with the main duct; the extra-pulmonary bronchi with the few larger branches of the duct, which coalesce to form it; the intra-pulmonary bronchi with the inter-lobular and intra-lobular branches; the bronchioles, or smallest bronchi, with the intermediate ducts; the infundibular and alveolar passages with the lumen of the alveoli of the gland; and the squamous epithelium of the one with the glandular epithelium of the other.

If we follow the mammalian trachea towards the lung, we find that, before reaching it, it divides into two bronchi, the right and the left. After entering the substance of the lung, these divide again and again dichotomously, to form the first or larger inter-lobular, intra-pulmonary bronchi. These subdivide, in like manner, to form lobular bronchi, each of which enters a lobule of the lung. Within the lobule the bronchus divides into several smaller branches, the bronchioles, each of which terminates in an infundibular passage, surrounded by the air vesicles of the lung.

The Structure of the Trachea.—The trachea, or windpipe (*Fig. 88*), is a tube consisting from within outwards of the following layers:—

(1.) The *Mucosa*, lined internally by a layer of ciliated epithelium (*a*), which is always stratified; *i.e.*, there are several layers of cells, the superficial of which alone bear cilia. Those immediately below them are younger cells, which in time will, as the superficial ones require replacing, reach the surface and acquire cilia; the most deeply placed cells are the germinal or proliferating ones, by the division of which the supply is maintained. Here and there, amongst the ciliated, will be found goblet cells. Beneath the epithelial lining there is found, in the human trachea, a very distinct basement membrane, of a clear homogeneous appearance. In chronic bronchitis, when the surface has become denuded of epithelium, this membrane often becomes enormously thickened from the irritation to which it is constantly exposed. In animals, such as the cat and rabbit, the membrane is very much thinner. Outside the epithelium there is a narrow layer of adenoid tissue (*b*), with a proportion of intermingled elastic fibres; and outside this a very distinct elastic layer (*c*). The fibres of this run longitudinally, and the outer

surface of the layer indicates the junction of the mucosa and submucosa.

(2,) The *Submucosa*, having a basis of ordinary connective tissue, with a considerable elastic element, and a varying amount of adenoid tissue, in the form of scattered masses.

The elastic tissue is present in the form of fibres, which are especially in evidence near to the elastic layer previously described. The submucosa also contains blood-vessels (*e*), nerves, and lymphatics (*f*), mucous glands, some non-striated muscle, and cartilaginous rings.

The mucous glands (*d*) are compound saccular glands, whose alveoli lie in the submucosa, and whose ducts extend through the mucosa, to open into the lumen of the trachea. The alveoli are lined by a single layer of columnar shaped, glandular, epithelial cells, the nuclei of which are situated near to the attached ends, especially when the gland is in a loaded condition. The amount of granularity of the cells, and their size also, depend on whether they are charged with mucin, or whether secretion has already taken place. The ducts are lined by cubical epithelial cells, in a single layer, between which and the ciliated epithelium on the surface there is a gradual transition where they meet.

The function of the gland is to secrete mucin, which is poured out on the surface, and moved by the cilia towards the pharynx, carrying with it any particles of foreign matter which may have entered the trachea and adhered to its wall.

The cartilaginous rings (*h*), varying from fifteen to twenty in number, are situated in the outer part of the submucosa, outside the glands, and entirely surround the trachea, except where they are deficient posteriorly. They are shaped more or less in the form of a horse-shoe. The gap between the ends is bridged across by the trachealis muscle, and outside the whole the connective tissue is condensed to form a general fibrous investment, each ring being in addition united to its neighbour by a continuation of the perichondrium.

The trachealis muscle is composed of bundles of non-striped fibres, which pass, for the most part transversely, between the ends of each cartilaginous hoop. Outside the transverse fibres, a few longitudinally placed ones may be seen. This muscle may be regarded as the remains of the original muscular wall of the alimentary canal, from which, as has already been stated, the lung is, in the first place, a diverticulum. The greater part

of the muscle has, so to speak, been displaced by the cartilage rings.

The mucous glands at this, the posterior aspect of the trachea, have their alveoli placed outside the trachealis muscle, their ducts piercing it to reach the inner surface of the tube.

Vascularisation of the Lung.—The trachea divides at its lower extremity into a bronchus for each lung. These, as they enter the organ, divide dichotomously again and again, to form the interlobular bronchi, running between the lobules of the lung. Each *intra-pulmonary bronchus* (Fig. 89A) is accompanied by a branch of the *pulmonary artery* (C) on one side, and of the *pulmonary vein* (B) on the other, the three tubes being enveloped in fibrous tissue, the *peribronchial connective tissue* (K).

The lung is supplied with venous blood from the right ventricle of the heart by the pulmonary artery, which enters it with the main bronchus, and, like it, subdivides again and again, the two remaining in close apposition throughout. When the terminal bronchus, or *bronchiole*, expands into the air cells or alveoli, the branch of the pulmonary artery accompanying it breaks up, to form a network of capillaries in the walls of the alveoli, beneath the epithelium. From these networks we have originating the pulmonary vein, containing arterial blood. The vein accompanies the bronchus in precisely the same way as the artery (the two, however, always being placed on opposite sides of the air tube), eventually pouring its blood into the left auricle of the heart, from whence it is transmitted, through the ventricle, to the systemic or longer circulation.

In a section through any part of the lung tissue, are to be seen, scattered over the surface, the open mouths of the sections of the bronchi, each with a blood-vessel on either side of it, a smaller one, the pulmonary artery, and a larger, the pulmonary vein. The three tubes are invested with a supporting fibrous sheath, continuous with the septa of fibrous tissue between the lobules. In addition, however, to the pulmonary artery and vein, the lung is supplied with blood by the bronchial vessels. The *bronchial artery* rises from the aorta, and, penetrating the lung at its root, accompanies the bronchi, and is distributed to the walls of the blood-vessels, the fibrous tissue of the bronchial passages, the interlobular septa, and the sub-pleural connective tissue. The blood is returned by the *bronchial veins* to the right vena azygos and left superior intercostal, by them to the

FIG. 89.

SEMI-DIAGRAMMATIC REPRESENTATION OF INTRA-PULMONARY BRONCHUS, WITH PULMONARY ARTERY AND VEIN, AND PERI-BRONCHIAL CONNECTIVE TISSUE $\times 20$.

- A.—Bronchus.
- B.—Pulmonary vein.
- C.—Pulmonary artery.
- D.—Bronchial vessels and nerve.
 - e.*—Epithelial lining of bronchus.
 - f.*—Adeno-elastic layer.
 - g.*—Muscularis mucosæ.
 - h.*—Mucous glands.
 - i.*—Cartilage plates.
 - j.*—Lymph follicular tissue.
- K.—Peri-bronchial connective tissue.

FIG. 89 a.

T.S. SMALL INTRA-PULMONARY BRONCHUS OF LUNG OF KITTEN,
STAINED WITH HÆMATOXYLIN $\times 20$.

- A.—Bronchus.
- B.—Pulmonary vein.
- C.—Pulmonary artery.
- D.—Bronchial vessels and nerve.
 - e.*—Epithelial lining of bronchus.
 - f.*—Adeno-elastic layer.
 - g.*—Muscularis mucosæ.
 - h.*—Mucous glands.
 - i.*—Small cartilage plate.
 - j.*—Lymph follicular tissue.
- K.—Peri-bronchial connective tissue.

Fig 89.

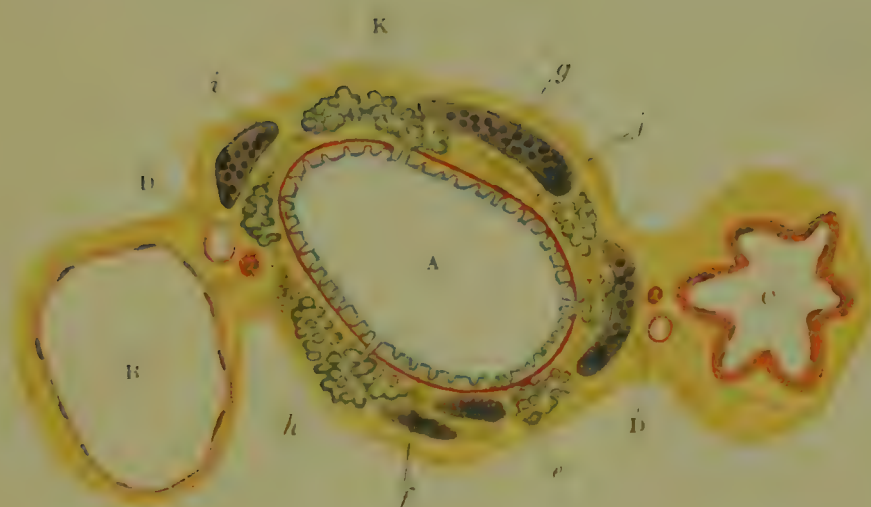
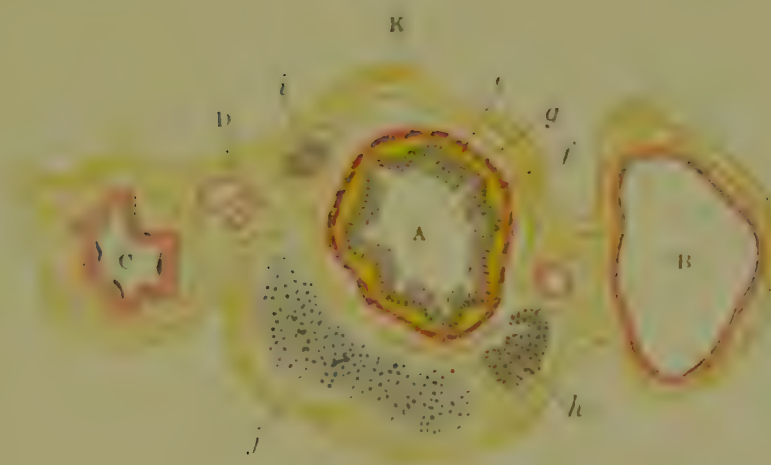
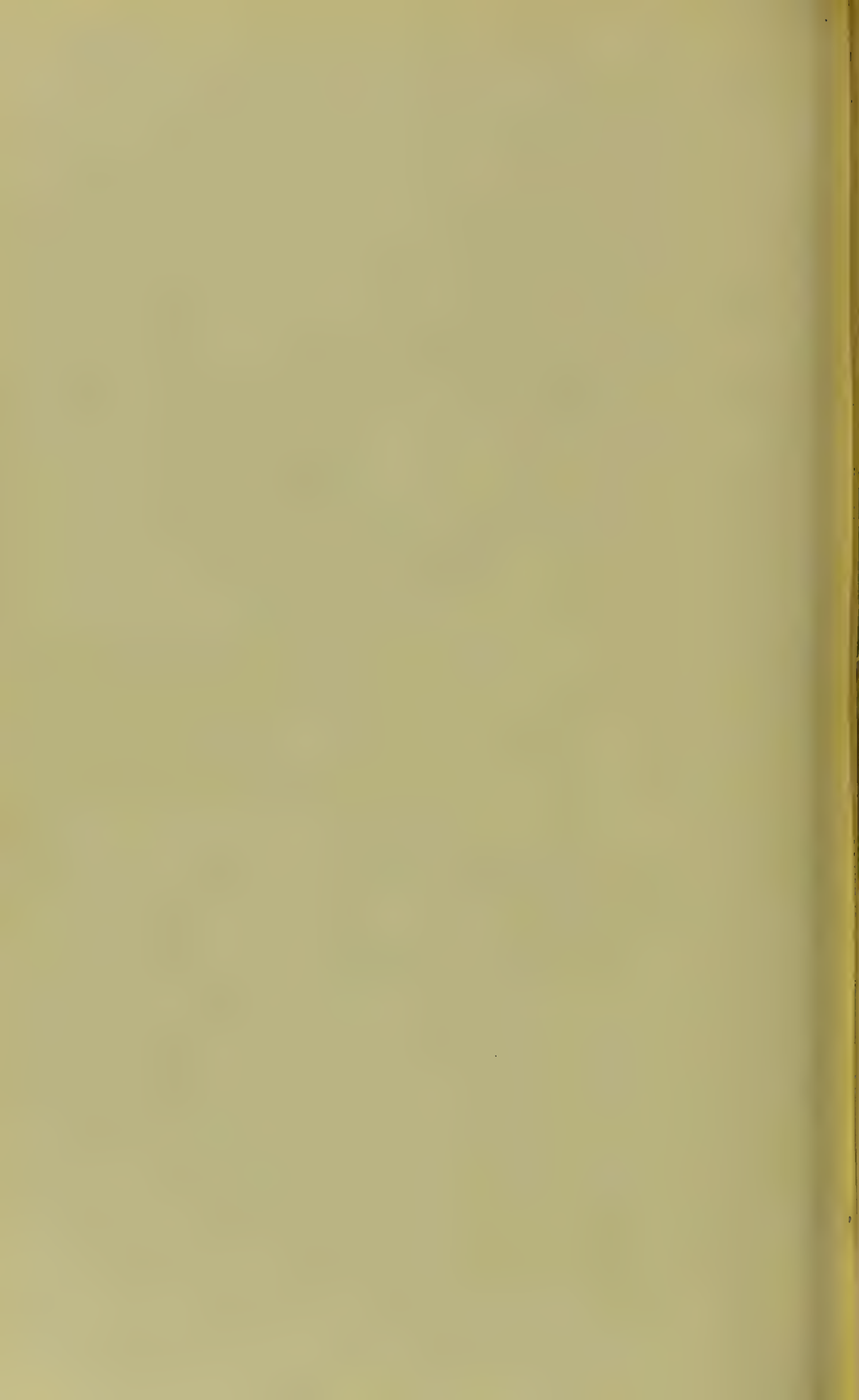


Fig 89a.





large veins at the root of the neck, and thence to the right side of the heart, where it mixes with the venous blood from the body generally. In the lung, the main bronchial vessels lie in the fibrous tissue around the bronchi, and are double in number; that is to say, a bronchial artery and vein (D), accompanied by a bronchial nerve, lie on either side of the bronchial tube. Thus, the pulmonary arterial vessels bring venous blood to the air cells of the lung, there to be oxygenated and returned in that condition to the heart; the bronchial arterial vessels supply the tissues of the lung itself with arterial blood.

But the two systems are not entirely separate from each other, for the bronchial branches which supply the mucous membrane of the air passages, end in capillaries which are in communication with the pulmonary veins, which thus return part of the bronchial supply. In this way obstruction to the return of blood by the pulmonary veins leads to congestion and catarrh of the bronchial mucous membrane.

Fibrous Tissue Framework of the Lung.—Before proceeding to describe the arrangement of parts in an interlobular bronchial passage in greater detail, it may be well to refer shortly to the general arrangement of connective tissue which obtains in most glandular organs; for though, in many cases, the arrangement may appear, at first sight, peculiar to the special organ under consideration, yet, as a matter of fact, it is, more or less, on the same plan throughout.

Organs, such as the lung, liver, and salivary glands, are enveloped with a layer of connective tissue, forming a general investment or capsule, which sometimes receives a special name. Thus, in the case of the lung, it is called the pleura; in that of the liver, the capsule of Glisson. The capsule varies somewhat in structure in each case, but, as a general rule, may be said to consist of white fibrous connective tissue, with a small elastic element, and contains blood-vessels, lymphatics, and nerves. From this general investment, fibrous septa are sent inwards, dividing the organ into lobules, and termed interlobular septa. At the point where the main artery enters the organ, and the vein and duct leave it, the fibrous investment sends in a prolongation of its tissue, which surrounds these tubes, and supports them in their ramifications. This supporting sheath is in continuity with the interlobular septa wherever it meets them; and, in fact, the latter may be regarded as ramifications of the

connective tissue sheath sent in round the vessels and duct, which extend through the substance of the organ, and come in contact with the general investment again on its inner surface.

Precisely the same plan of arrangement obtains in the case of the lung (*Fig. 96*). The general investment is called the pleura (*a*); the fibrous sheath, sent in round the vessels and duct (bronchus), the peri-bronchial connective tissue (*b*); the tissue separating the lung into lobules, continuous on the one hand with the bronchial sheath, and on the other with the pleura, the interlobular septa (*c*).

The *pleural sac*, like the pericardial, consists of two layers—a visceral covering the surface of the organ itself, and a parietal lining the cavity in which it is placed—the two being continuous over the root of the lung, in the same way that the two layers of the pericardium become continuous over the roots of the great vessels. The sac is lined by polygonal, squamous, epithelial cells, amongst which are numerous orifices, or *stomata*, guarded by smaller epithelial cells, communicating with the lymphatic spaces in the connective tissue outside.

If a vertical section of the visceral layer of the pleura be studied, it will be seen to consist of two layers—an outer, fibrous, and an inner, loose one, containing many lymphatics. The lymphatics are in connection, on the one hand, with the cavity of the pleural sac, through the stomata already referred to; and on the other, with the lymphatics of the lung generally, *i.e.*, those of the inter-lobular septa, the peri-bronchial tissue, and the connective tissue around the alveoli. The lymph from the pleural sac, after traversing these channels, passes through the bronchial lymphatic glands, thence to the thoracic lymph channels, and so into the general circulation.

Structure of an Intra-pulmonary Bronchus.—The main features of difference between the trachea and the bronchi consist in the breaking up of the tracheal cartilaginous ring into three or more pieces of cartilage, and in the appearance in the bronchi of a muscular coat which has been called the *muscularis mucosæ*.

From without inwards occur the following layers (*Fig. 90*):—

(1.) The *Mucosa** possesses internally a layer of stratified ciliated epithelium (*a*), similar to that of the trachea, except that it is thrown into longitudinal folds, comparable to those formed by the intima of an artery. This may be due in the

human subject to *post-mortem* contraction of the muscularis mucosæ, but in some animals, *e.g.*, the cat, the ridges and furrows remain after the lung and trachea have been filled with fluid and distended to a greater extent than during life. Beneath the epithelium there is not, as in the case of the trachea, a broad, homogeneous membrane, though a considerably thinned representative of it can be seen in the larger bronchi of the human lung. Outside the epithelial layer we come to a narrow, adeno-elastic one, consisting of adenoid tissue, with longitudinal elastic fibres running through it. Beyond this is an exceedingly definite narrow layer of non-striped muscle circularly arranged (*b*). It is often called the muscularis mucosæ, and certainly appears to occupy the position of one. It probably, however, has the same morphological derivation as the non-striped muscle of the trachea.

(2.) *The Submucosa** consists of ordinary connective tissue of the areolar variety, and merges into the general fibrous investment of the bronchus at its periphery. It contains the mucous glands (*c*) whose ducts, after piercing the muscularis mucosæ, open on the lumen of the tube; here and there, collections of adenoid tissue, forming lymph follicular masses; blood-vessels, lymphatics and nerves; and, in its outer part, the cartilaginous plates (*d*). These plates are often three in number, and are situated in the outer part of the coat. As the bronchi become smaller, they tend to be more irregular in size, number, and position.

The glands are similar to those found in the wall of the trachea. In the cat, their ducts usually open into the permanent furrows between the longitudinal folds of epithelium. The cartilage plates are of ordinary hyaline cartilage.

On each side of the bronchus, and surrounded by the same fibrous sheath, is a large blood-vessel—on the one side, the pulmonary artery, and on the other, the pulmonary vein.

Outside the cartilage plates, usually between the bronchus and the pulmonary vessels, are found the bronchial vessels and nerves; a bronchial artery, vein, and nerve, occurring on each

* The words Mucosa and Submucosa are here used as they generally are in descriptions of the structure of a bronchus. If the muscularis mucosæ is the representative of the main muscular coats of the alimentary canal, it would seem to follow that the tissue, lying outside it, corresponds with the fibrous peritoneal investment of the intestine.

FIG. 90.

T.S. INTRA-PULMONARY BRONCHUS OF CAT'S LUNG (INJECTED), STAINED
WITH HÆMATOXYLIN $\times 40$.

- a.*—Ciliated epithelium.
- b.*—Muscularis mucosæ.
- c.*—Glands.
- d.*—Cartilage.
- e.*—Bronchial vessels.
- f.*—Lung alveoli.
- g.*—Peri-bronchial tissue.

FIG. 91.

T.S. INTRA-PULMONARY BRONCHUS (HUMAN), STAINED WITH PICRO-
CARMINE $\times 300$.

- a.*—Ciliated epithelium.
- b.*—Adeno-elastic layer.
- c.*—Muscularis mucosæ.
- d.*—Mouth of mucous gland.
- e.*—Adenoid tissue.
- f.*—Cartilage.
- g.*—Mucous gland.
- h.*—Submucosa.

Fig. 90.

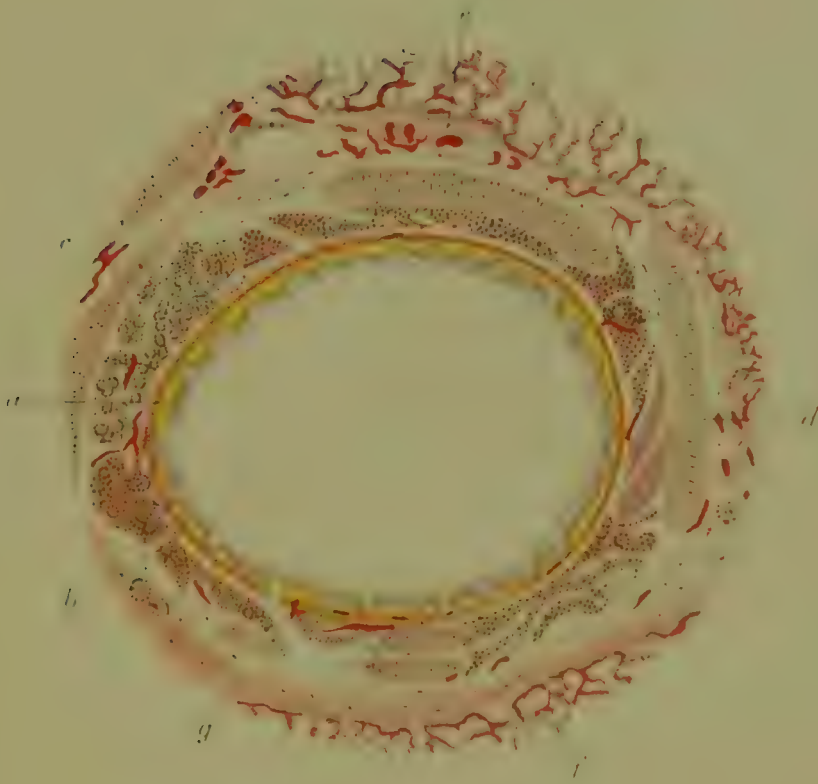


Fig. 91.



side, and thus being double the number of the pulmonary vessels.

Structure of the Terminal Bronchioles and Air Vesicles.—The bronchi, in becoming smaller, lose their cartilaginous plates, and come to consist of a layer of stratified, ciliated epithelium, surrounded with a muscularis mucosæ, and this again with peri-bronchial connective tissue, containing blood-vessels and lymphatics, mucous gland acini, and lymph nodules (*Fig. 89 a*). If one of these smaller tubes be followed still further towards its termination, the ciliated epithelium first becomes a single layer, and then loses its cilia, becoming simply columnar or cubical in character; the muscularis mucosæ persists, though in a more scattered form; the peri-bronchial tissue is greatly reduced in quantity, and does not contain mucous glands or lymph nodules. In fact, we have now a tube, lined by cubical epithelium, surrounded by circular non-striped muscle fibres, the whole being invested with a comparatively thin fibrous sheath. Let us now trace this terminal bronchial tube or bronchiole into the alveolar “glandular” portion, that part of the organ in which aëration of the blood takes place (*Fig. 94*).

A bronchus or bronchiole (A), at its termination, opens into an *infundibular passage* (B), which is merely the continuation of the bronchial tube in a perforated condition. Its wall has a similar structure, being lined with cubical epithelial cells, surrounded with non-striped muscle, and invested with fibrous tissue; but its continuity, as a complete tube, is interrupted by the presence of large round, or oval apertures, each of which leads into an *infundibulum* (C).

In the figure there will be seen seven of these openings in transverse section, and two of them in surface view from the centre of the tube. Each of these openings is continued, in its infundibulum, as an *alveolar passage* (D). This passage is so called because its wall is formed by a succession of *alveoli* or air cells (E). These alveoli may be regarded as rather shallow cups placed side by side, their adjacent walls in close apposition to each other, the mouths of the cups being turned towards the alveolar passage. An infundibulum, therefore, is one of these systems of cups, arranged round an alveolar passage, and it opens by one of the rounded apertures into the bronchiolar tube, or, as it is called in this situation, the infundibular passage.

In the wall of this passage, the cubical epithelium and the non-stripped muscle are both still present; in the alveoli, the epithelium has become squamous, and the non-stripped muscle is absent. It is as though, in the pushing out of an infundibulum from the wall of the bronchiole, the cubical epithelium, lining it, had become flattened and expanded in order to cover the increase of surface required; the pushed out portion passing between the strands of muscle fibres, which do not, therefore, invest it with a covering; the fibrous tissue, however, with its capillary network, forming an external covering to the ex-vagination. If we further conceive an additional or secondary series of ex-vaginations to take place from the inner surface of the first, resulting in the formation of the air vesicles, we obtain a very fair idea of the terminal air passages.

The *alveolar wall* consists of three parts:—

(1,) A layer of squamous epithelial cells; (2,) A network of capillary blood-vessels; (3,) Elastic tissue.

The epithelium consists of large, flattened, squamous cells with a polygonal outline, and frequently without nuclei (*Fig. 93 B*). Between these are, here and there, to be seen smaller granular cells (*g*), which are always nucleated, and which stain readily with ordinary reagents.

The connective tissue, in which the network of capillaries is imbedded, consists of a meshwork of elastic fibres, continuous with the connective tissue surrounding the bronchiole, and separating the infundibula from each other.

The septum (*D*) between two alveoli is covered on both its sides with a layer of squamous epithelium, the two layers being continuous over its free edge. Between the two layers is the capillary network and elastic tissue.

The lung in the *fœtus* is unexpanded, as no inspiration of air has yet taken place; the blood of the child being oxygenated and relieved of CO_2 in passing through the placenta. The epithelium, lining the unexpanded air cells, differs from that of the air cells of the mature lung in being also unexpanded, that is, it is composed of cubical "glandular" looking cells instead of flattened squames. The original glandular nature of the lung is indeed very apparent, the bronchioles representing the terminal intra-lobular duct, and ending in alveoli in a distinctly racemose manner.

Examine the following specimens:—

(1.) *T. S. Trachea of child, stained with hæmatoxylin. F. or B. (Fig. 88.)*

First hold the specimen up to the light, and with the naked eye make out the more deeply stained cartilaginous ring, in the form of a horse-shoe; and the more lightly stained mucosa and submucosa on its inner surface. It is well, in fact, to do this as soon as the specimen is upon the slide, and before it is covered, as the parts do not always hold well together, especially if the section is thin.

With the low power, determine the relative positions of the various parts. Find the layer of epithelium (*a*) next to the lumen. Even under this power it is not difficult to recognise the bright, refractile line, indicative of the free border of the cells, and the cilia placed upon it. Notice that the nuclei of the epithelium are in more than one layer, indicating that the cells are two or three deep. Observe, here and there, amongst the ciliated cells, one which has become a goblet or mucin-forming cell. Now find one of the ducts opening on to the surface, and trace it to the glands (*a*), in the submucosa. Each duct, just before it opens on to the surface, will be seen to become considerably dilated. The detail of the structure of the glands themselves can hardly be seen under this power, but the nuclei of the cells of an alveolus can be seen forming a deeply-stained ring towards the periphery. Note also the rather large lumen of the alveoli. The glands are collected into groups, the alveoli often imbedded in adenoid tissue, the whole mass appearing more deeply stained than the connective tissue of the submucosa in which it lies.

Return to the surface. Recognise again the epithelium; note the deeply stained line in its lower part, caused by the close apposition of the nuclei of the cells of its deepest or germinal layer. Beneath, or outside this, the clear basement membrane, slightly stained with the hæmatoxylin, is readily seen. Outside this is a narrow, lightly stained layer of adenoid tissue, with elastic fibres intermingled (*b*). Still passing from within outwards, look for another layer of about the same width—the layer of longitudinally running elastic fibres (*c*)—in this section cut transversely. They are much more readily seen, however, as a well-defined layer, under the low power, in a longitudinal section of the trachea. Here they form a narrow band of bright, refractile dots, representing the elastic fibres cut transversely.

FIG. 92.

S. LUNG OF DOG, STAINED WITH NITRATE OF SILVER $\times 50$.

- A.—Bronchiole.
- B.—Peri-bronchial connective tissue.
- C.—Pulmonary blood-vessel.
- D.—Infundibulum.
- E.—Walls of alveoli.

FIG. 93.

S. LUNG OF KITTEN, STAINED WITH NITRATE OF SILVER $\times 300$.

- A.—Bronchiole.
- B, E.—Alveoli.
- C, D.—Alveolar walls.
 - g.*—Small granular cells.
 - h.*—Alveolus cut in plane above level of floor.

Fig. 92.

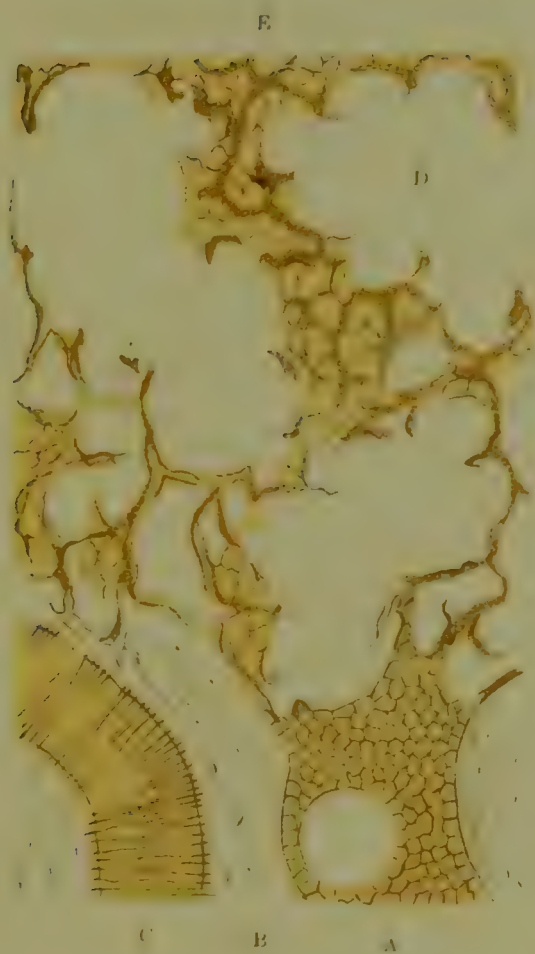
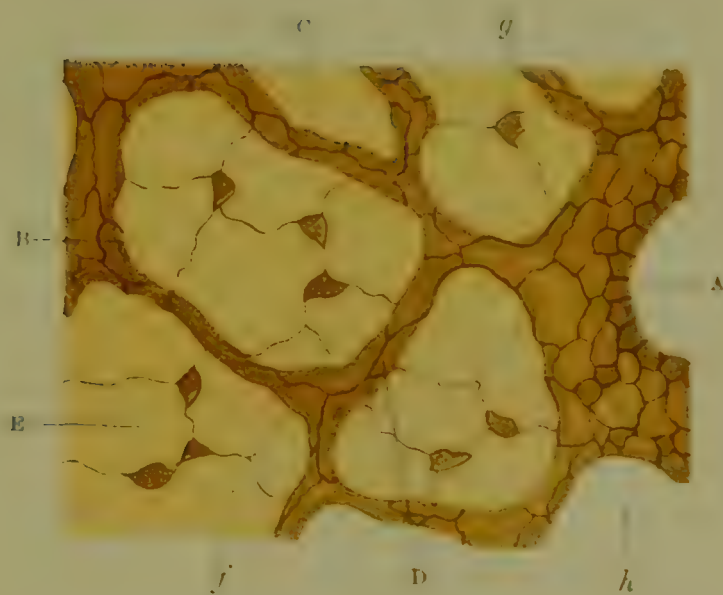


Fig. 93.



Now look for the ring of hyaline cartilage (*h*), a much more deeply-stained mass, situated in the outer part of the sub-mucous coat, and forming the greater part of the thickness of the tube. Notice the perichondrium (*g*), or fibrous investment of the cartilage itself, and outside this the general fibrous investment of the trachea.

Look at the posterior part of the section, between the ends of the hyaline cartilage, and identify the bundles of non-striped muscle; the inner, cut longitudinally, and seen as bands passing between the ends of cartilage; the outer, as isolated patches of transversely divided fibres. Now look carefully for gland alveoli, placed outside the muscle, and find, if possible, a duct piercing the latter. Occasionally, about this region, small isolated pieces of cartilage may be seen imbedded in the fibrous connective tissue. Under the high power, study first the ciliated epithelium. The cilia, the refractile band, the many nuclei, can all be much more distinctly and easily made out than before. Trace the epithelium into one of the gland ducts, and notice the gradual transition between it and the single layer of cubical cells, forming the duct itself. Next, find an alveolus of a gland, and study its epithelium. Notice that the lumen of the alveolus is quite distinct, and, in many cases, very large. Observe the cells of the alveolus. They are columnar in shape, and may be in different stages of granularity, according to their stage of functional activity. If large, and charged with mucin, they are clear, lightly stained, and transmit the light readily; if small, from the mucin being discharged, they are granular, and stain more deeply. The cells, as a rule, transmit the light fairly well, and in this way form a contrast to the more opaque appearance presented by a serous gland. Note, too, the position and shape of the nucleus. If the cell is distended with mucin, it is pushed away, so to speak, to its attached end, and flattened in the plane of that end. This alters after secretion, the nucleus becoming round and approaching the centre of the cell. Study the rest of the sub-mucous coat, with its blood-vessels, lymphatics, etc. Notice the very considerable amount of elastic tissue, which stands out very distinctly in the form of bright, refractile dots, the transverse sections of the fibres.

Examine now the peculiar, broad, homogeneous basement membrane, the adeno-elastic layer beneath it, and the still more definitely elastic layer separating the last from the

submucosa. Examine the cartilaginous ring and trachealis muscle.

(2,) *L. S. Trachea of child, stained with hæmatoxylin.*

Examine a longitudinal section of the trachea of a child, in order to see—

(a,) The elastic layer cut in longitudinal section.

(b,) The transverse sections of the cartilaginous rings.

(3,) *T.S. Intrapulmonary bronchus and vessels, lung of cat, stained with hæmatoxylin and eosin, or picro-carmin. F. or B. (Fig. 90. See also Fig. 91.)*

It is an advantage to have the tissue injected, as its permeation with a solidified gelatine mass greatly helps to keep the parts of the sections in position, if they are cut in gum; otherwise they have a great tendency to fall apart, especially if the section is thin. This does not, of course, apply to specimens cut in paraffin.

Look at the mounted section: first, with the naked eye, and identify the bronchial opening, the largest of the three, with a smaller opening on either side; those of the pulmonary artery and vein respectively. The whole "bronchial passage" presents a contour somewhat similar to that shown in *Fig. 89*. Now place the specimen under the low power (*Fig. 90*). Find first the lumen of the bronchus, and observe the peculiar arrangement of the layer of ciliated epithelium (a) surrounding it. Note the regularly arranged longitudinal folds (here cut transversely), and that it is only the summit of each fold which takes part in the formation of the lumen of the tube. The epithelium is not of uniform thickness throughout, being thicker on the top of the folds than it is in the furrows between them. Such an appearance is certainly against the idea that these ridges, in this animal at least, entirely disappear on distension of the tube. It is suggestive of the membrane presenting this arrangement constantly during life, distension of the bronchi resulting in a temporary separation of adjacent folds, but not in their obliteration; relaxation of the distending force causing a closer approximation of them, so that only their tops take part in limiting the bronchial lumen.

Outside the epithelium, though not in contact with it, except where it lines the furrows, look for the circular layer of non-striped muscular fibres (b). In a hæmatoxylin specimen this is frequently stained of a somewhat pink colour, and even

under this power its fibrillated appearance and rod-shaped nuclei, stained blue with the reagent, may be made out. Look for interruptions to the continuity of the layer here and there, due to the passage through it of the ducts of the mucous glands, to open into the furrows between the folds of the epithelial layer. If the specimen has been treated with eosin, as well as hæmatoxylin, the muscle is picked out by the eosin, and stained an eosin red, the nuclei being still blue.

Between the muscular layer and the epithelium, identify the adeno-elastic layer as a very light unstained band, running up into a small papilla-like point, where it forms the central core of a fold, and becoming attenuated, or disappearing, where the muscular coat touches the base of the epithelium in the furrows.

Now examine the structures outside the muscular layer. First identify the masses of gland alveoli and adenoid tissue (*c*). They present a granular, rather deeply stained appearance, due to the colouration of the nuclei of the cells with hæmatoxylin. Under this power little more of the structure of the glands can be made out, as they are a good deal smaller than those found in the wall of the trachea. Notice, here and there, the injection mass in the blood-vessels in this situation. Lying outside the masses of gland tissue, are to be seen the plates of cartilage (*d*), varying in size, shape, and number, in different sections, uniformly stained, and presenting a hyaline appearance. Observe outside the cartilage plates, a varying thickness of connective tissue, separating the bronchus from the parenchyma of the lung, by which it is surrounded. Trace this peri-bronchial tissue round the tube, till it becomes continuous with that enveloping one of the pulmonary vessels. Follow it round this vessel on to the bronchus again, and then round the vessel on the opposite side, till the starting point is once more reached, noting its continuity throughout. Around the vessels it represents the adventitia, or outer coat. Note the relative size of the two vessels, and the more or less irregular lumen of the artery from the contraction of its walls. Observe that the walls, both of the artery and vein, are considerably thinner than in the case of an ordinary blood-vessel.

Now look for the bronchial vessels and nerves (*e*). These are easily found in the fibrous tissue outside the cartilage plates, usually between the bronchus and the large vessels; two vessels, an artery and a vein, accompanied with a nerve, being placed on each side respectively.

FIG. 94.

DIAGRAMMATIC REPRESENTATION OF TERMINATION OF A BRONCHIOLE
IN THE LUNG.

- A. —Bronchiole.
- B.—Infundibular passage.
- C.—Infundibulum.
- D.—Alveolar passage.
- E.—Alveolus.
- f.*—Connective tissue.
- g.*—Muscle fibres.
- h.*—Epithelium.

FIG. 95.

S. INJECTED LUNG OF HUMAN FŒTUS × 250.

Pulmonary artery, injected red.
 „ vein „ blue.

Fig. 94.

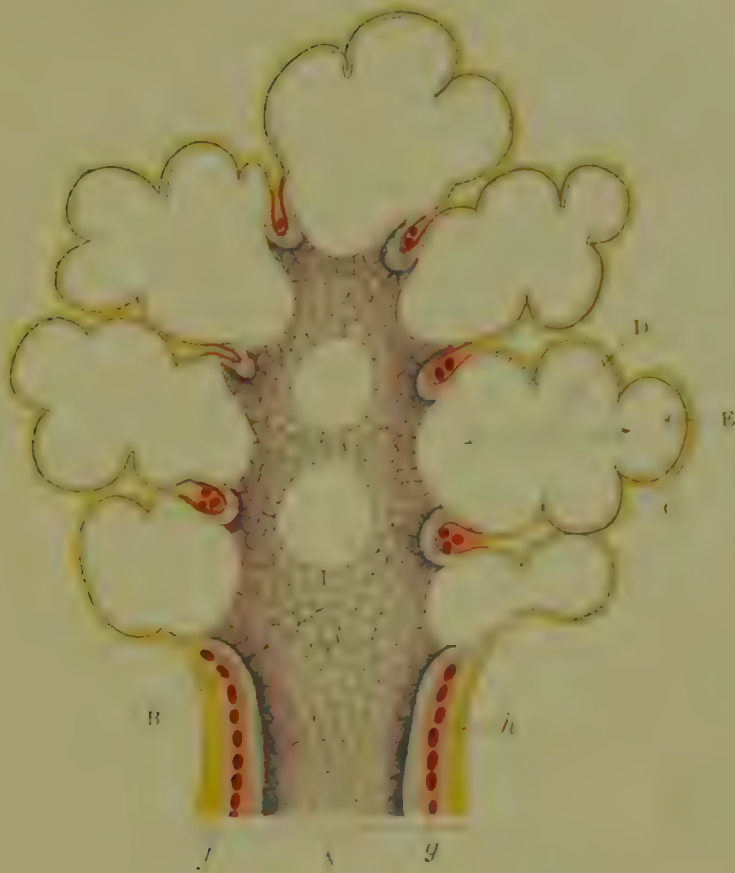
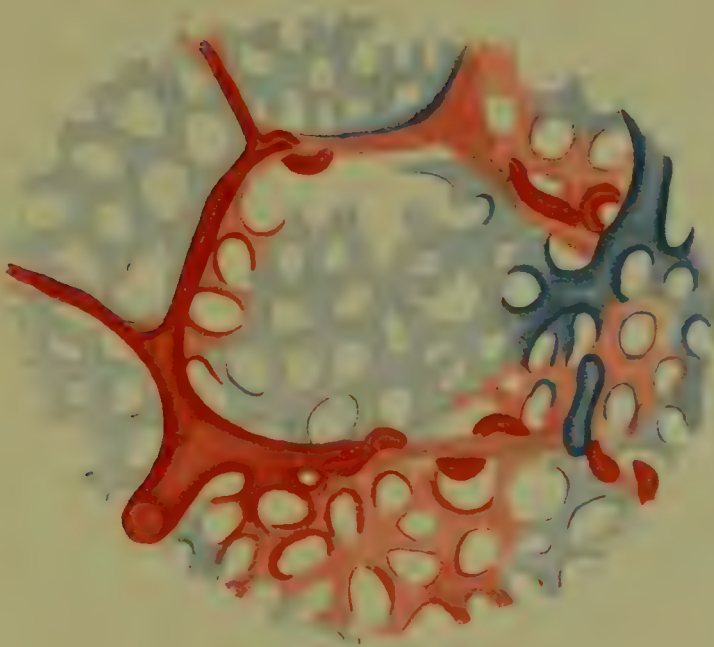


Fig. 95.



Examine briefly the lung tissue (*f*) beyond the "bronchial passage." Note the peculiar network formed by the injected walls of the alveoli seen in section; here and there, sections of small arteries and veins, filled with injection mass, may be seen. Glance over the rest of the field. Perhaps smaller bronchi, with their accompanying vessels, may be occasionally distinguished. Find one of these smaller bronchi (*Fig. 89a*), from which the cartilaginous plates have almost, if not entirely, disappeared. Note the structure of its walls. Internally, a layer of ciliated epithelium (*e*), still stratified and convoluted, as in the case of the medium sized tube just examined, but not so regularly; outside, an adeno-elastic layer (*f*), surrounded with a well marked muscularis mucosæ (*g*). In the fibrous tissue beyond, look for some still remaining mucous glands (*h*), and usually one or more considerable masses of lymph follicular tissue (*j*). The presence of this is a special feature of the smaller bronchial tubes. On each side observe the large pulmonary and smaller bronchial vessels as before (*C, B, D*).

Under the high power (*Fig. 91*), examine the structures found in the wall of the medium sized bronchus. First study the epithelium (*a*), and determine its ciliated stratified character. Trace its connection with the ducts (*d*) of the mucous glands (*g*), as in the case of the trachea. Find the adeno-elastic layer (*b*), in which the transversely cut elastic fibres stand out clearly as bright refractile dots. Besides the elastic tissue, note the small, round, deeply stained nuclei of the lymph corpuscles, and sections of capillary blood-vessels. Observe how this layer varies in thickness, according to the position it occupies with regard to the folds of epithelium. Observe the circular layer of non-striped muscle (*c*). In thin sections the individual fibres can be made out. The deeply stained, rod-shaped nuclei are very evident under this power.

In the submucosa notice, first, the mucous glands (*g*), which vary somewhat in appearance, according to their particular stage of secretion, as in the trachea. Now look for a lymph follicular mass (*e*). The structure of one of these follicles may be described shortly as follows: It is usually a rounded or oval collection of adenoid tissue, permeated with a capillary network. It is surrounded with a lymph sinus, which is lined with epithelioid cells, and traversed by septa, which bear blood-vessels, or consist merely of fibrous tissue, thus forming

either a vascular or purely fibrous connection between the follicle and the tissue outside. In an injected specimen, such as this, the capillary network of the follicle is easily seen as a network of injection material, traversing the mass of lymph corpuscles.

These latter are small round cells, for the most part smaller than a white blood corpuscle. They have, for their size, very large nuclei, with a minimum amount of peri-nuclear protoplasm. The adenoid reticulum cannot be readily made out in an ordinary specimen of this tissue, owing to the densely packed lymph corpuscles in its meshes, nor can the peripheral lymph sinus be seen. Examine the cartilage plates, observing the hyaline character of the cartilage, and the transition between the cells at its periphery, and the connective tissue cells of the perichondrium. Find the bronchial vessels and nerve. The nerve is easily recognised under this power. Note its fibrous investment, perineurium, and the transverse sections of the nerve fibres, with the stained axis cylinder as a dot, in the centre of them. Find again, and examine with this power, the smaller bronchus.

(4.) *Section of fetal lung, stained with hæmatoxylin. B. (Fig. 96.)*

To study the disposition of the connective tissue framework of the lung, a section of foetal lung may be examined. Such a lung is more appropriate for the purpose than an adult one, as it is unexpanded, and the fibrous tissue septa are, therefore, more evident. When the lung becomes expanded by inspiration, the septa necessarily become thinned out considerably, and their connections are not nearly so diagrammatically shown.

Under the low power, the parts, figured in the drawing, will be made out at a glance. The portions of lung tissue, mapped out by the fibrous septa (*c*), are the lobules, and each may be considered a lung in miniature. A lobule consists of the terminations of one, two, or occasionally more bronchioles (*g*). Find one of these bronchioles. Some are cut transversely, others longitudinally, in which case their connection with the alveoli may be apparent. As shown in the figure, they are more deeply stained than the alveolar portion of the parenchyma, or appear to be so from the closer apposition of the nuclei of the cubical or columnar cells lining them. There is shown at (*g*) one of these bronchiolar divisions, with the alveoli in connection with it, showing the resemblance to the fundamental structure of a racemose secreting gland.

Now look at the interlobular septa (*c*), more lightly stained than the lobules they separate. Observe, especially at the junctions of the septa with each other, the bronchial tubes (*d*), and pulmonary vessels (*e*); just as in the interlobar and interlobular septa of a gland, such as the submaxillary, we find its ducts and vessels. The bronchus is readily distinguished by its lining of columnar epithelium, with deeply stained nuclei. The fibrous tissue, investing it, is the peribronchial connective tissue (*b*), and is continuous with the connective tissue of the septa meeting at this point. Trace the septa towards the surface, and notice their continuity with pleura (*a*).

In both pleura, septa, and peribronchial tissue, look for lymph spaces and channels of various form. Though not obviously so in the section, these are, in reality, in continuity with each other, and it will, therefore, be readily understood how foreign particles, entering the peribronchial lymphatics through the bronchial wall (or the alveolar lymphatics, with which these are continuous, through the alveolar wall) come to be deposited, as in coal miners' phthisis, in every part of the fibrous tissue framework of the lung. Under the high power verify these points, noting particularly the pleura with the lymph spaces in its deeper layer, continuous with those in the interlobular septa; the structure of the tubes in the bronchial passages; the structure of the intralobular divisions of the bronchi, and the alveoli proceeding from them, lined with cubical epithelium. Observe the connective tissue between the alveoli. This is very distinct in the foetal lung; in the expanded lung it is much less appreciable.

(5.) *Section of injected lung of cat, unstained. B.*

Study the vascularisation (pulmonary) of the alveoli in an injected lung of some animal, such as the cat.

Fig. 95 is from the lung of a human foetus, in which the pulmonary artery has been filled with red injection, and the vein with blue.

As a rule, the best method of preparation is to inject the lung of a freshly killed animal from the pulmonary artery, and force it through the organ into the pulmonary vein. To inject successfully with two colours requires a great deal of practice, and is necessarily very much more uncertain in the result than with one.

Examine under the low and high powers respectively. The

FIG. 96.

S. OF HUMAN FŒTAL LUNG, SHOWING THE RELATIONS OF THE DIFFERENT PARTS OF THE FIBROUS FRAMEWORK TO EACH OTHER, STAINED WITH HÆMATOXYLIN $\times 15$.

- a.*—Visceral layer of the pleura.
- b.*—Peri-bronchial connective tissue.
- c.*—Interlobular connective tissue.
- d.*—Small intrapulmonary bronchi.
- e.*—Pulmonary artery.
- f.*— „ „ vein.
- g.*—Bronchiole within a lobule, expanding into,
- h.*—Alveoli.

FIG. 97.

SEMI-DIAGRAMMATIC REPRESENTATION OF WALL OF ALIMENTARY CANAL.

- | | |
|---|--|
| <p>A.—Mouth and œsophagus.</p> <p>B.—Stomach { 1.—Cardiac end.
2.—Pyloric „</p> <p><i>a.</i>—Mucosa.</p> <p><i>b.</i>—Submucosa.</p> <p><i>c.</i>—Muscular coat.</p> <p><i>d.</i>—Peritoneal coat.</p> <p><i>e.</i>—Internal circular muscular coat.</p> <p><i>f.</i>—External longitudinal muscular coat.</p> <p><i>g.</i>—Glands of Brunner.</p> <p><i>h.</i>—Solitary gland.</p> <p><i>i.</i>—Auerbach's nerve plexus.</p> | <p>C.—Small intestine.</p> <p>D.—Large intestine.</p> <p>E.—Rectum.</p> <p><i>j.</i>— { Villi of } Duodenum.</p> <p><i>k.</i>— { Villi of } Jejunum and ileum.</p> <p><i>l.</i>—Glands of Lieberkühn.</p> <p><i>m.</i>—Muscularis mucosæ.</p> <p><i>n.</i>—Skin beyond anus.</p> <p><i>o.</i>— „ „ mouth.</p> <p><i>p.</i>—Tooth.</p> <p><i>q.</i>—Salivary Gland.</p> <p><i>r.</i>—Mouths of gastric follicles.</p> <p><i>s.</i>—Pyloric sphincter.</p> |
|---|--|

Fig. 96.

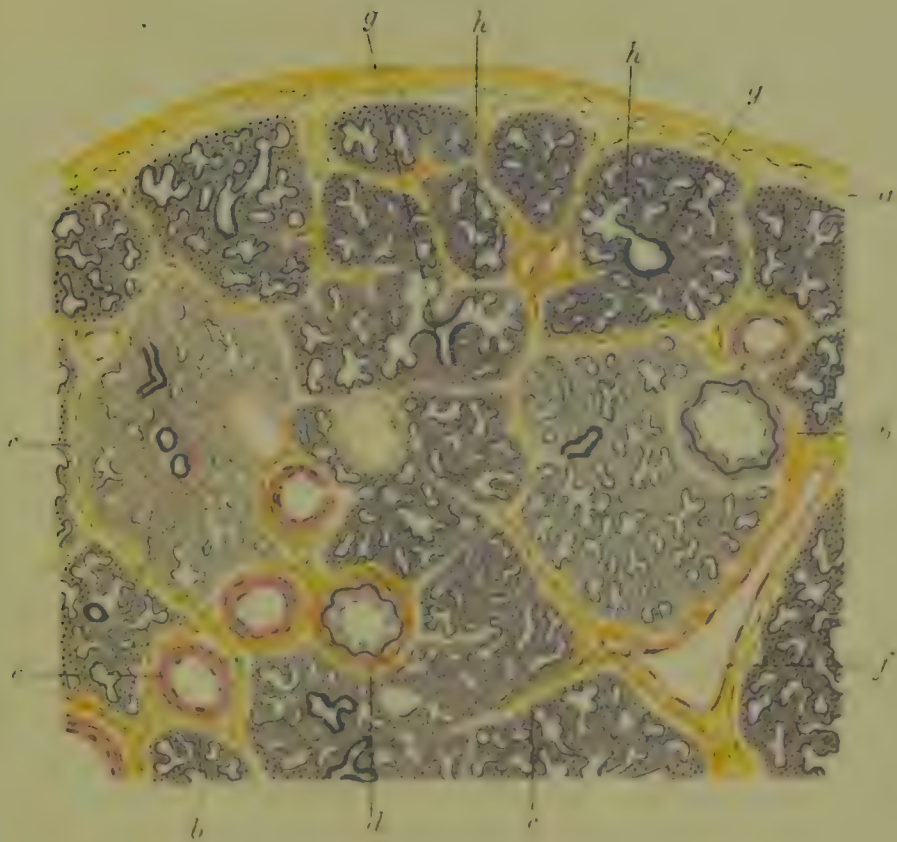
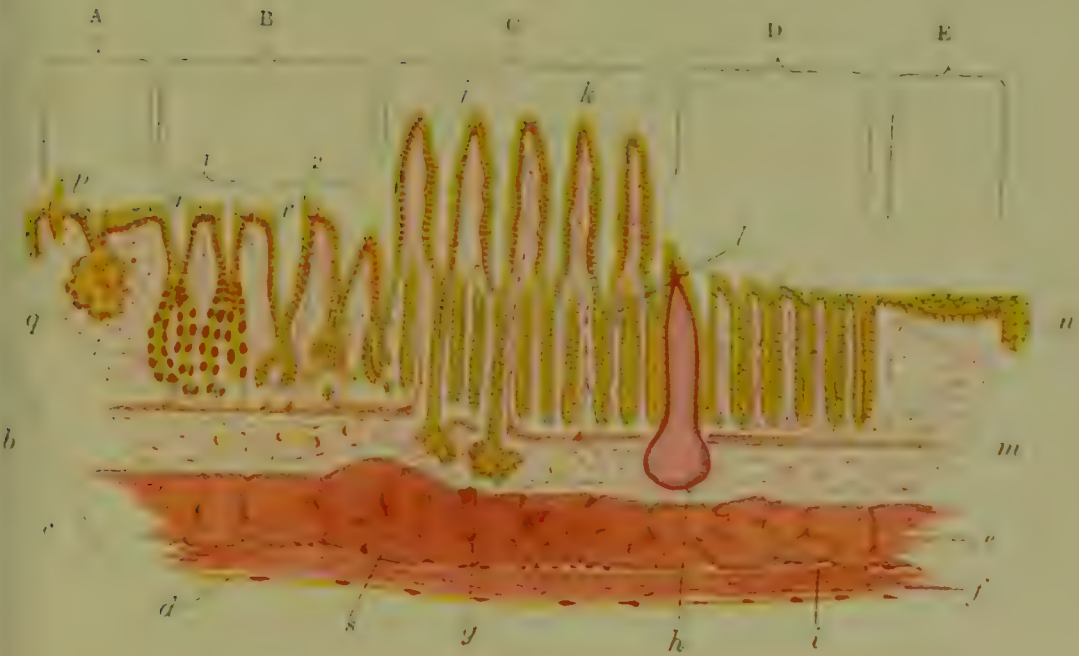


Fig. 97.



section is so easily understood, that it scarcely requires special explanation.

Observe the larger vessels, here and there, in the peribronchial connective tissue, their lumen filled with injection mass, and the smaller ones running amongst the alveoli. Notice the size and shape of these, and, under the high power, especially the character of the capillary network lying in their walls. Note the smallness of the meshes of the network. Many of these rounded or polygonal alveoli are seen as spaces outlined with the alveolar walls in section; many show the network of vessels in their floor. This will be easily understood by a reference to *Fig. F*, in which the alveoli are represented as shallow cups

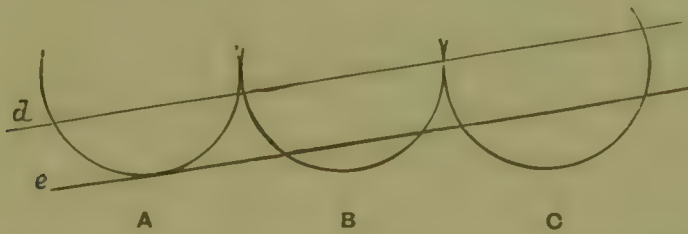


FIG. F. Diagram of alveoli of lung. A, B, C, alveoli; *d*, *e*, lines of supposed section through them.

placed side by side. Suppose a section is taken so as to include the parts of the cups between the lines, *d* and *e*. Then, in looking down on such a specimen through the microscope, the alveolus, A, will be cut so as to show the floor; B will be cut so as to show all but the lowest part of the floor of the cup; while C will show no floor at all, but only the sides.

(6.) *Section of lung of dog, stained with silver nitrate. F. (Fig. 92.)*

(7.) *Section of lung of kitten, stained with silver nitrate. F. (Fig. 93.)*

Use (6) for examination under the low power especially, and (7) for the high.

The lung of the dog is especially suitable for showing the disposition of the infundibula, and the bronchioles terminating in them. Under the low power (*Fig. 92*) observe, first, the outlines of the alveoli (E), and then the much larger spaces with scalloped outlines (D), the alveolar passages of the infundibula. The scalloped margin is due to the projection inwards of the septa, between the alveoli, which form the infundibular wall. Even under the low power, in a well stained specimen, the silvered outlines of the epithelial cells lining the alveoli may be

to some extent made out. Now look for sections of the bronchial tubes (A), which are rendered very evident from the deep staining of their epithelial lining, and the pulmonary vessels accompanying them (C), supported by comparatively unstained fibrous tissue (B).

Under the high power, examine the kitten's lung for the alveolar epithelium. Find a part where the floor of an alveolus is seen from the surface (*Fig. 93*). Note the epithelial plates (B), outlined by the nitrate of silver, which has been reduced by the cement substance between them. Here and there, between the plates, note the small granular darker cells (*g*), their nuclei, however, not being revealed by the reagent. Examine the septa (D) between adjacent alveoli. From the greater depth of tissue looked through, these necessarily appear much darker than the alveolar floor. If the section has included the free edge of the septum, the outlines of the epithelial cells passing from one side to the other will be seen.

Now look for one of the small bronchiolar tubes, such as is figured in the drawing (A). Observe the outlines of the cubical epithelial cells lining it, and trace this epithelium towards the alveoli. Note that the transition between the two forms of cells is not sudden, the cubical becoming gradually more and more intermingled with squamous as the alveoli are approached. A little care is required to find a part showing the transition well.

APPENDIX TO CHAPTER X.

METHODS OF PREPARATION.

1. Trachea.—Harden human (young child) and cat's trachea in 2 per cent. solution of chromic acid for a week or ten days, and complete in spirit. Cut T. and L.S.'s of each in gum, stain in picro-carmin or hæmatoxylin, and mount in Farrant or balsam; or stain in bulk in borax-carmin, and cut in paraffin. Only a portion of T.S. child's trachea can, however, be conveniently cut in paraffin.

2. Intrapulmonary bronchus.—(a.) Remove the lungs and trachea of a cat, fill with 2 per cent. solution of chromic acid, allowing them to float in same fluid the while. The fluid can be conveniently introduced into the trachea from a pipette holding about 20 c.c. When the lungs are fully distended with the fluid, ligature the windpipe, and place the whole in excess of the solution used for a week or two. At the end of that time the tissue should be fairly well "fixed," and when the lung is cut into cubes of half an inch, the pieces should not collapse. Complete in spirit. The lung should have been so cut as to show the bronchus with the artery on one side, and the vein on the other, in transverse section. Select a cube showing this well; stain in bulk in borax-carmin, and cut in paraffin. (b.) Inject the lungs of a cat with carmine gelatine mass from the pulmonary artery, by the continuous air-pressure method; remove, allow to cool, cut into suitable cubes, and harden in alcohol. Select a piece showing an intrapulmonary bronchus cut transversely; cut in gum, stain in hæmatoxylin, and mount in balsam. The object of the injection here is to keep the parts together.

3. Fœtal lung.—Harden pieces in Müller, or Muller and spirit, cut in gum, stain in picro-carmin or hæmatoxylin, and mount in Farrant or balsam; or stain in bulk in borax-carmin, and cut in paraffin.

4. Injected lung.—Carefully inject the lungs of a cat through the pulmonary artery with carmine gelatine mass by continuous air pressure. The pressure must be kept low, as less force is required than for systemic injection. It is well to begin with a pressure of less than one inch, and increase it very gradually. The left auricle should be cut to allow of the escape of blood, which may advantageously be washed out of the lung, previous to injecting the gelatine, with salt solution. Cool, cut into pieces when set, and harden in spirit. Cut thick sections in gum, and mount in balsam.

5. Silvered lung.—Fill the lungs of a small dog and a kitten with .25 per cent. solution of nitrate of silver, through the trachea. It is important to get as much of the air replaced as possible. This can be facilitated by squeezing

the lungs gently in one hand from time to time, as the fluid is being run in. When the lungs are filled, ligature the trachea, and place in 50 per cent. of spirit for half an hour. Now replace the nitrate of silver with spirit, and suspend in same for a few days till hardened. Cut in pieces, and preserve in spirit. Cut in gum, and expose to light, when reduction takes place. Mount in Farrant's solution. The dog's lung is especially serviceable for showing the infundibular arrangement, and that of kitten for the epithelium of the terminal alveoli; but, if necessary, the dog's lung will do for both quite well.

CHAPTER XI.

*THE ALIMENTARY CANAL.**THE TONGUE ; THE TEETH ; THE SALIVARY GLANDS.***GENERAL PLAN OF CONSTRUCTION OF THE CANAL.**

THE alimentary canal commences at the mouth, and terminates at the anus. Its epithelium, with the exception of that lining the mouth, the œsophagus, and the termination of the rectum, is wholly of hypoblastic origin. The large glands (the salivary glands, the liver, and pancreas) are special out-growths of the epithelial layer, surrounded with connective tissue and blood-vessels. Before describing the structure of the various parts of the canal it will be well to mention, to some extent in detail, its general plan of construction. It is lined internally by *epithelium*, which differs in character in different regions, according to the function which it has to subserve. In the mouth and œsophagus, where this is largely mechanical, it is of the nature of stratified squamous epithelium ; in the stomach and intestine, where its function is absorptive and secretory, it consists of a layer of "glandular cells" of the columnar type ; and the same holds good with the large intestine, and the rectum to the margin of the anus, where it becomes protective in function, and here again it is of the nature of stratified squamous epithelium. Beneath the epithelium, throughout the whole of the canal, is a layer of *connective tissue*, which is modified in the various parts with a view to the function of these parts. In the mouth, œsophagus and rectum, it is of the nature of ordinary connective tissue ; that is, it consists of white fibres, with a certain proportion of elastic fibres and connective tissue cells. In the

stomach, the small and the large intestine, it is more of the nature of adenoid tissue. Throughout, this connective tissue contains blood capillaries, which are developed to an especial extent in those parts of the tract lined by active glandular epithelium. It contains also in some parts special muscular and lymphatic arrangements, which will be dealt with when the individual parts are more fully described. At the surface of this connective tissue, forming in sections a line between it and the epithelium covering it, there is a "basement membrane"; in this case consisting of a thin layer of condensed tissue—a layer made up partly of connective tissue and partly of flattened connective tissue corpuscles. The membrane is thus not a complete one, but composed merely of a surface condensation of connective tissue. Beneath the layer of connective tissue there is one of non-striped *muscle*, a narrow layer, composed for the most part of fibres arranged circularly and longitudinally, the internal ones circularly and the external, longitudinally. This layer is absent in the mouth, and imperfect in the œsophagus, but fairly complete throughout the rest of the canal. These three parts—the epithelium, the connective tissue, and the muscularis mucosæ—constitute together the **mucosa** of the alimentary canal.

Outside the mucosa is found the **sub-mucous layer** of connective tissue, which consists throughout of typical areolar tissue. It is a broad layer, loosely arranged, containing large blood-vessels, nerves, and lymphatics, and connects the mucous coat with the subjacent muscular one. It is distinctly loose, and admits of free movement within certain limits between the mucosa and the muscular coat; so free that the former readily forms folds upon the lumen of the canal, when the latter is at all contracted. It corresponds with the deeper part of the cutis vera of the skin, which allows of free movement of the epithelium and more condensed fibrous tissue beneath it, upon the subjacent structures.

Outside the submucosa we have the **muscular wall** of the canal. Except in part of the œsophagus, this consists throughout of non-striped muscle, which is specially developed at certain points to form sphincters. It is arranged in two layers, a thicker internal circular coat, and an outer thinner longitudinal one. Between the two is a small amount of connective tissue, containing Auerbach's nerve plexus.

Surrounding the canal throughout the greater part of its extent we have the **peritoneal covering**, consisting of a delicate

layer of connective tissue, invested with simple squamous epithelium.

Fig. 97 shows the relations of these parts, throughout the whole of the canal, diagrammatically.

Secretion and absorption by the alimentary canal.—*Secretory epithelium*, in its simplest form, consists of a layer of cells with a stratum of vascular connective tissue immediately beneath it. The cells withdraw from the lymph, with which the tissue is bathed, those substances necessary for the formation of the secretion which they pour out upon their free surface. Any of the serous sacs will serve as an illustration of this simple arrangement. Usually, however, when the secretion is at all a special one, the arrangement is much more complicated. The first differentiation consists in an inpushing of the epithelium in the form of a tubular or saccular process into the tissue beneath it. Instances of these *simple tubular* or *saccular glands* are found in Lieberkühn's follicles of the intestine of mammals, and in the simple glands of the frog's skin respectively. These are termed simple tubular and saccular glands, the name being descriptive of the shape of the invagination. They are represented diagrammatically in *Fig. G*. When the secreting epithelium has advanced to this stage of specialisation, its cells usually possess a more or less cubical or columnar shape, and it is termed, glandular epithelium. The next step towards complexity consists in the division of the simple tube or saccule, so that a compound gland is the result. An instance of a *compound* gland of the *tubular* variety, in its early stage of development, is found in

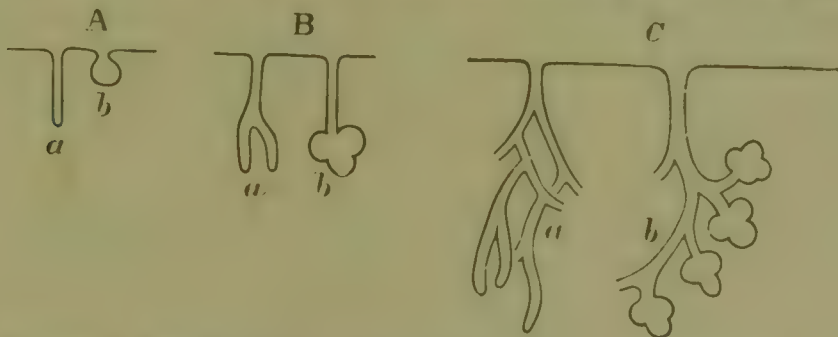


Fig. G.—Diagram of different classes of glands: *A*—Simple: *B*—Commencing complexity; *C*—Compound; *a*—Tubular; *b*—Saccular.

the glands of the stomach, to be shortly described. Here, the lower part of the tube becomes divided, and constitutes the secreting part of the gland, the upper part remaining single and

being termed the duct. A later stage of development is shown in the case of the testis, where we have the duct dividing again and again into smaller subdivisions before the terminal secreting portion of the gland is reached. An instance of a *compound* gland of the *saccular* variety is found in one of the salivary glands, *e.g.*, the submaxillary. Here we have again a repeatedly subdividing duct, the ultimate subdivisions of which terminate in clusters of small saccules or alveoli. These various kinds of glands will be described in greater detail as they occur; at present it will be sufficient to draw attention to the general principle of construction which is common to all. They all consist of an inpushing of epithelium (or in the case of glands of the alimentary canal an outpushing); this inpushing may remain simple, as it frequently does when the glands are spread over a large surface, as in the case of the intestine, or it may become compound when a considerable surface is required, but little space is available, as in the case of the salivary glands. Whether the gland is simple or compound, its epithelium rests upon a basement membrane, separating it from the connective tissue around it. The basement membrane is, however, usually of the nature merely of a surface condensation of this tissue. The surrounding connective tissue supports a blood capillary network. The substances required for the production of the secretion of the gland pass from the blood capillaries into the lymph spaces of the connective tissue, through the basement membrane, into the gland cells. Here, they are sometimes combined in a new form, *i.e.*, rearranged, or, without such rearrangement, they may be passed on into the lumen of the gland tubule or saccule, as the case may be, and thence poured out upon the surface either directly in the case of simple glands, or, indirectly, through the duct or ducts in the case of compound glands.

The stomach, small and large intestines, are *absorptive* as well as secretory, and the process of absorption is performed by that part of the epithelium between the invaginations which constitute the glands. These intervening portions of epithelium are in the small intestine specially developed in connection with the connective tissue beneath them to form *villi*. This absorptive epithelium is of the columnar glandular variety. In this case, the stream passing through the epithelial cells is in exactly the opposite direction to that which is taken in the case of a secreting gland.

Here, some of the contents of the alimentary canal enter the free end of the cell, traverse it, and pass through the basement membrane beneath, into the lymph spaces in the connective tissue, and thence into the blood capillaries, or, in the case of fat absorbed in the small intestine, into lacteal vessels.

The structure of the alimentary canal and the glands opening into it may be considered in the following sections: *The tongue; the teeth; the salivary glands; the œsophagus; the stomach; the duodenum; the pancreas; the liver; the ileum and jejunum; the large intestine; the rectum.*

THE TONGUE.

The tongue is composed chiefly of a series of muscles of the voluntary striated variety, so arranged as to facilitate the various movements required. These muscles are separated from each other, and supported, as usual, by connective tissue, continuous with that surrounding the organ generally; and the whole is enveloped with a layer of stratified squamous epithelium, continuous with that lining the rest of the cavity of the mouth. Over the whole of the superior surface of the tongue this epithelial layer is raised into projecting papillæ, which have a corresponding core of vascular connective tissue. These papillæ are of three kinds: filiform, fungiform, and circumvallate. In each the fibrous tissue core is split up at its extremity into a series of secondary papillæ, which may vary in number from five to thirty. The epithelium bears corresponding secondary projections, in the case of the filiform papillæ. The superficial surface of the fungiform and circumvallate papillæ is, however, smooth (*Fig. 99*).

The *filiform papillæ* are the most numerous, and are found covering the dorsum of the tongue, with the exception of the edges and tip. They are conical in shape. They are shown in vertical section in *Fig. 101*. The epithelium covering them is divisible, like that of the mouth generally, into two layers, a deeper softer one, the stratum Malpighii, and a superficial harder one, the stratum corneum. It is prolonged at the apex of the papilla into pointed processes corresponding with the subdivisions of the connective tissue core it covers.

The *fungiform papillæ* are found especially at the tip of the tongue and along the edges of the upper surface. They are not narrow and pointed like the preceding, but thicker, broader,

FIG. 98.

V.T.S. TONGUE OF DOG, STAINED WITH HÆMATOXYLIN \times 5.

- a.*—Epithelium of dorsum of tongue, raised into papillæ.
- b.*—Subepithelial connective tissue.
- c.*—Epithelium of inferior surface.
- d.*—Inferior longitudinal muscle.
- e.*—T.S. lingual artery and nerve.
- f.*—Radiating muscle.
- g.*—Superior longitudinal muscle.
- h.*—Median raphé.
- i.*—Extrinsic muscle.

FIG. 99.

DIAGRAMMATIC REPRESENTATION OF PAPILLÆ OF TONGUE.

- A.—Filiform papillæ.
- B.—Fungiform „
- C.—Circumvallate papillæ.
 - a.*—Epithelium lining mouth.
 - b.*—Fibrous tissue papilla, with secondary papillæ upon it.
 - c.*—Epithelium covering papillæ.
 - d.*—Serosus gland (Ebner's) opening into vallum round circumvallate papilla.

FIG. 100.

SECTION OF RABBIT'S TONGUE, INJECTED \times 50.

- a.*—Epithelium of dorsum.
- b.*—Subepithelial tissue.
- c.*—Muscular substance.

(The loops of blood-vessels running up from the subepithelial tissue, apparently into the epithelial layer, mark the positions of the fibrous tissue papillæ, which are not otherwise indicated in the drawing.)

Fig. 98.



Fig. 99.

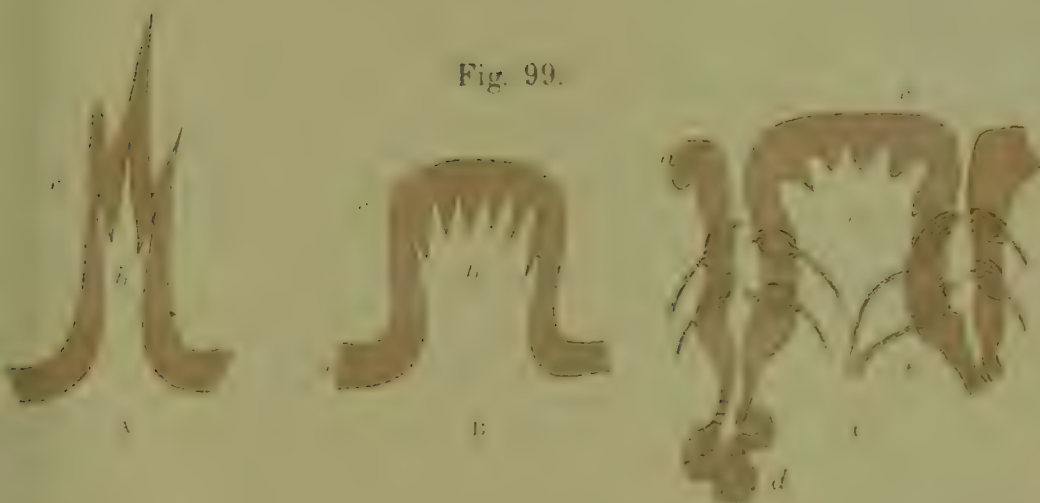


Fig. 100.



blunt on the surface, and less projecting. Like the filiform, they possess a core of connective tissue, which runs up into the epithelium covering it, in the form of secondary papillæ. The epithelial layer, however, is not subdivided at its surface; below, of course, it fills in the intervals between the secondary fibrous papillæ.

The *circumvallate papillæ* are confined to the posterior part of the dorsum of the tongue, where they are arranged in the form of a V, with the apex pointed backwards. They are about eight to twelve in number. They differ from the two preceding forms in being surrounded with a vallum or ditch, along the sides of which numerous taste buds occur. The papilla itself (*Fig. 99 C*) resembles the fungiform in shape, but is larger. It possesses secondary fibrous papillæ, but its epithelial covering is smooth on the surface. It only projects slightly above the level of the rest of the epithelium of tongue, as the greater part is sunk beneath the surface. Serous glands—the glands of Ebner—open into the vallum.

Glands of the Tongue.—The tongue possesses glands of two kinds, mucous and serous. The serous glands open, as above stated, into the valla round the circumvallate papillæ; the mucous, much the more numerous, along the sides of the tongue, and at its base.

Taste buds of the Tongue.—These peripheral terminations of the nerves of taste, are found on the fungiform papillæ, on each side of the valla of the circumvallate papillæ (*Fig. 102*), on the soft palate, and the posterior surface of the epiglottis. They are small oval bodies, consisting of cells which have been differentiated from the general epithelial lining of the mouth. *Fig. H* represents one of these diagrammatically. The cells forming a taste bud are of two kinds (1,) *Outer protective cells*. These, in their arrangement may be likened to the staves of a barrel. In shape they are long fusiform cells, with a nucleus, causing a central bulging. They surround (2,) the *gustatory cells*. These are narrower, fusiform nucleated cells, whose central ends are connected with fibres of the gustatory nerve, and whose periph-



Fig. 11.
Diagram of Taste-bud :
a.—Sheathing cell; *b.*—
Rod Cell; *c.*—Epithelium
lining vallum; *d.*—Gusta-
tory pore, through which
the hairs of the central rod-
cells *b*, project; *e.*—Nerve
filaments.

ral ends terminate in hairs, which project through the open end of the barrel—the *gustatory pore*. (A third kind of cell is also described—the “subsidiary.” These are more nearly allied to the central gustatory or rod-cells than to the protecting, but possess a larger nucleus and more perinuclear protoplasm. They are, to some extent, interspersed among the rod-cells, but for the most part are collected round them, and to the inside of the outer protecting cells.) One of these oval taste buds extends the whole depth of the epithelial layer in which it occurs; the gustatory pore, opening on the surface, and the other end, where the nerve fibrils enter it, reaching the subjacent connective tissue.

Examine the structure of the tongue in the following sections:—

(1.) *V.T.S. Tongue of cat or dog, stained with hæmatoxylin. B.*

Examine the specimen first with the naked eye (*Fig. 98*).

Note the general shape of the tongue in transverse vertical section. It is somewhat triangular. The base of the triangle, its longest side, is uppermost, and corresponds with the dorsum or superior surface of the tongue. The two sides are below, and represent the sides and under surface of the tongue. Observe the deeply stained epithelial investment (*a*), and the lightly stained layer of connective tissue beneath it (*b*). The body of the organ is muscular, and even with the naked eye the different systems of muscular fibres may be distinguished. Look first for the *median raphé*, a vertical fibrous septum running antero-posteriorly, and showing as a narrow streak in the middle of the specimen (*h*). On either side of this, in the mid-region, are to be seen muscular fibres radiating laterally (*f*). Along the superior surface of the tongue, immediately beneath the subepithelial connective tissue, are to be seen the transverse sections of the superior longitudinal muscle (*g*). Along the inferior sides of the triangle, with the same relative position to the subepithelial connective tissue in this region, are to be seen the transverse sections of the inferior longitudinal muscle bundles (*d*). Look now for the system of fibres running vertically on either side the median raphé, passing between the longitudinal bundles above and below to end in the subepithelial connective tissue. On either side of the middle line, below the level of the fibres radiating from the raphé, look for the lingual artery cut transversely (*e*.)

Under the low power (*Fig. 101*), make a more detailed examination. Observe the papillæ (*a*) covering the upper surface of the tongue, and extending a very little way upon the sides. For the most part, these papillæ are of the filiform variety, amongst which, however, fungiform ones may be seen scattered here and there. Towards the edges of the tongue, note that they become, for the most part, fungiform. Beneath the epithelium, observe the connective tissue layer (*b*) faintly stained, containing blood-vessels and nerves, and beneath this, study the arrangement of the muscular fibres. These fibres belong to two different systems: (1,) bundles cut transversely (*d*) and, therefore, running longitudinally along the dorsum of the tongue; (2,) Bundles cut longitudinally (*c*), alternating with the previous ones which they thus cross at right angles. These fibres run vertically, and some of them may be traced to the inferior surface of the tongue where they pierce the inferior longitudinal muscle, and terminate in the subepithelial connective tissue. Observe the median raphé in section and the muscular fibres radiating from it. Notice the directions these radiating fibres take. The superior ones curve upwards and pass between the bundles of the superior longitudinal muscle; the lower ones curve downwards and pass between the bundles of the inferior longitudinal muscle; the middle ones pass directly outwards. Observe how these radiating fibres terminate with some abruptness where they meet the more lightly stained subepithelial connective tissue. Now look at the point of junction of the two sides of the tongue below. It is not in reality a pointed junction, but the two sides curve inwards and upwards, so as to form an inferior fissure, reaching to the commencement of the median raphé. In this inferior fissure, look for strands of one of the extrinsic muscles of the tongue (*Fig. 98 i*). On each side of the upper part of the fissure, beneath the lower radiating fibres, look for transverse sections of the lingual artery and nerve (*e*). The artery is easily recognisable from its deeply stained media, and affords an excellent illustration of the structure of the smaller arteries.

Little more is to be made out with the low power. Note that there are four systems of intrinsic muscles: (1,) Superior longitudinal; (2,) inferior longitudinal; (3,) radiating from the raphé; (4,) passing vertically from the upper to the lower surface of the tongue.

Under the high power examine first the papillæ. Select one

of the filiform papillæ (*a*) and note the nature of the epithelium covering it. It is in two layers, a deeply stained Malpighian and a lightly stained or unstained corneous layer. Note the usual layer of germinal cells in the deepest part of the Malpighian layer. Observe the core of lightly stained connective tissue, with secondary papillæ sometimes shown upon it. After this, look for one of the fungiform papillæ, observing its blunt extremity and greater breadth. The circumvallate papillæ will be examined in another specimen. Now study the line of junction between the subepithelial connective tissue and the muscular tissue beneath it. The transversely cut fibres (*d*) of the superior longitudinal muscle appear to have no connection with the connective tissue, but to be contained in arches formed above by the edge of this tissue, and at the side by the fibres (*c*) of the vertical system which run into it. Study their mode of termination. At the point where they reach the connective tissue they become conical in shape, and terminate in what is virtually tendon—in white fibres which become lost amongst the rest of the subepithelial connective tissue. These muscle fibres, both in longitudinal and transverse section, should be studied as instances of ordinary striated muscle. Examine the epithelium of the under surface of the tongue, and notice that it is free from papillæ, and consists of stratified squamous cells arranged in two layers, as before.

(2.) *V.S. Tongue of sheep or man, stained with hæmatoxylin (for circumvallate papillæ). B.*

This is a section through one or more of the large circumvallate papillæ.

Under the low power observe the large bulbous circumvallate papilla, the greater part of it sunk beneath the level of the rest of the tongue. Examine the epithelium on each side of the vallum for taste buds. These are better seen in the next preparation, but can usually be made out in this, as small oval-shaped bodies, three or four in number on each side, stained rather less deeply than the rest of the epithelium. Now look at the lowest part of the ditch, and endeavour to trace its connection with the serous gland alveoli, embedded amongst the superficial muscular fibres of the tongue. The serous alveoli appear as small granular round bodies, forming a cluster amongst the strands of muscular fibres, and frequently the duct connecting them with the vallum can be seen in longitudinal section. Examine also with the high power.

(3.) *V.S. Papilla foliata of rabbit's tongue, stained with hæmatoxylin (for taste buds). F. or B. (Fig. 102.)*

The papilla foliata is a small leaf-like portion of epithelium, situated on each side of the rabbit's tongue, at its base. The epithelium in this area is thrown into a series of longitudinal folds, with furrows between them, and the taste buds are situated along the sides of the furrows. The section is taken vertically to the surface generally, and transversely to the line of the ridges, and thus gives the appearance of a number of papillæ placed side by side. It must be borne in mind, however, that these are not papillæ, but transverse vertical sections of ridges.

With the low power, observe the "papillæ," ten to fifteen in number, side by side; with depressions—the furrows—between them. Notice the two layers of the epithelium; the Malpighian layer stained deeply with the hæmatoxylin, and the narrow superficial corneous layer of flattened squames, frequently almost neglected by the reagent. This corneous layer is continued with the one beneath it into the furrows (*a*¹), but becomes much narrower in this situation, and fades away towards the lower part of them. Now look for the taste buds (*b*) in the epithelium bounding the furrows. They are much more distinctly seen in this specimen than the last. Next observe the connective tissue running up into secondary ridges beneath the epithelium, then the muscular substance of the tongue, and embedded in it, near to the surface, the alveoli of serous glands (*e*) opening by means of ducts (*d*) into the bottom of the furrows.

Find, again, a well defined taste bud, and put on the high power. Note the characters of a taste bud as far as they can be made out in such a section. They may be cut through their centre, in which case both the central cells and the sheathing are cut longitudinally; or, the knife may have passed to one side, in which case, only the sheathing cells may be cut. But in neither case is it easy to make out the characters of the individual cells very distinctly. The taste bud appears as an oval body, marked longitudinally by lines indicating the edges of the cells forming it, and here and there the nuclei of these cells are to be seen. Look for the gustatory pore; the small opening through which the hairs of the central cells project into the furrow. This will easily be seen in many of the taste buds examined, but not in all, as the section may not have passed through it. Examine the connective tissue forming the core of the ridge under in-

FIG. 101.

V.S. TONGUE OF DOG, STAINED WITH HÆMATOXYLIN \times 200.

- a.*—Filiform papillæ.
- b.*—Fibrous tissue.
- c.*—Muscle fibres cut longitudinally.
- d.*— „ „ transversely.

FIG. 102.

V.S. PAPILLA FOLIATA OF RABBIT'S TONGUE, STAINED WITH HÆMATOXYLIN \times 250.

- a.*—V.T.S. one of the ridges.
- a'*.—Furrow on each side of ridge.
- b.*—Taste buds.
- c.*—Nerves of taste to buds.
- d.*—Duct of serous gland, opening into furrow.
- e.*—Alveoli of serous gland lying amongst,
- f.*—Muscular fibres.

Fig. 101.



Fig. 102.



spection, and distinguish in it the nerve fibres (*c*) passing to the proximal end of the taste buds. These fibres may generally be recognised from the number of nuclei upon them. Now examine the serous gland alveoli. The structure of a serous gland will be described in detail and examined in connection with the large salivary glands, and it will therefore be sufficient to say here that they are compound saccular glands. The main duct of one of these glands opens into the bottom of a furrow, and its branches terminate in alveoli, lined by serous gland cells. The alveoli are small, the cells lining them granular; there is no perceptible lumen, and the nuclei are placed towards the centre of the cells. The ducts are lined with a single layer of cubical epithelium continuous with the stratum Malpighii at the bottom of the furrow. The student, in examining such a gland for the first time, is always more or less disappointed that its structural arrangement is not diagrammatically seen, and is apt to blame his specimens. But if he remembers for a moment that such a gland resembles somewhat a bunch of grapes, of which the main branch represents the chief duct, the stalks the smaller ducts, and the grapes themselves the alveoli; and imagines such a cluster cut through in any one plane taken at random; he will understand the improbability of a diagrammatic representation of the connections between the various parts resulting. He must be content to see cross sections of alveoli and ducts with often no apparent connection between them.

(4.) *V.S. Injected tongue of rabbit, unstained. B. (Fig. 100.)*

Note in this specimen the large vessels in the connective tissue layer (*b*) between the epithelium (*a*) of the dorsum of the tongue and the muscular substance (*c*), and the delicate branches sent from these into each of the fibrous papillæ, in which they break up into a fine capillary network. Observe that the vessels of the connective tissue layer are continuous with still larger ones in the muscular substance of the tongue. Note the network of capillary vessels running longitudinally between the muscular fibres, and giving off transverse branches to each other. This is one of the best situations for studying the vascularisation of striped muscle (*Fig. 47*).

THE TEETH.

A tooth consists of two parts—the *crown*, above the level of the gum; and the *fang*, situated below. The point of junction between the two is called the neck. The fang is sometimes single, or it may show two, three, or four subdivisions. The crown is covered with a cap of *enamel*; the fang with a thin covering of osseous tissue, the *crusta petrosa*. The centre of the tooth is hollowed out into the *pulp cavity*, which extends both into the crown of the tooth and the fang. The point of the fang is perforated to allow of the entrance of nerves and blood-vessels to the pulp cavity. The main part of the tooth is composed of *dentine* (Fig. 104 B). The dentine consists of a homogeneous matrix, in which lie the *dentinal tubules* (*c*). The inter-tubular matrix is calcified. The wall of the tubules is perhaps of the nature of elastin. These tubules lie close together, and give to the dentine a striated appearance when they are cut in longitudinal section. They radiate outwards from the pulp cavity to the periphery; the dentinal tubules in the fang thus having a different direction to those in the crown, and those in the middle of the crown to those at its sides. Those in the fang pass horizontally outwards, those in the central part of the crown vertically, and those at its sides are of course intermediate in direction. The dentinal tubules pursue a somewhat wavy course, and divide dichotomously in giving off lateral branches, which anastomose with those of neighbouring tubules, especially towards the periphery of the dentine. At the periphery of the dentine are to be seen the *inter-globular spaces*. In an unsoftened tooth they appear black, from the air they contain (*b*). They are irregular spaces, the sides of which are formed of a number of convexities inwards. They indicate small areas of imperfect calcification.

The dentinal tubules contain, in the recent state, the *dentinal fibres*, one to each tube. These fibres are branches of the odontoblast cells of the pulp, to be described immediately, and represent the terminations of the nerve in the tooth. It is these fibres which convey sensory impressions when the dentine of a decayed tooth is scraped.

Surrounding the dentine in the fang is the narrow layer of bone—the *crusta petrosa*. It differs from bone, however, in not possessing a system of Haversian canals.

The *enamel* (A) covering the crown of the tooth is the hardest structure in the body, and is composed almost entirely of inorganic material in the form of calcium salts. It consists of a series of calcified rods (*a*) with little cement substance between them, which are placed side by side, vertically to the surface of the enamel, and thus have of course a different direction at the sides of the crown from what they have at the top. These rods are hexagonal in transverse section; when cut longitudinally they show very fine transverse markings.

The alveolus of the jaw (*Fig. 103*) is hollowed out for the accommodation of the teeth, but a tooth is not in direct contact with the bony socket it occupies. Between the two is what is termed the *alveolar* or *periodontal membrane* (*c*¹), a vascular layer of connective tissue of some thickness, which serves to fix the tooth in position, and at the same time to act as a buffer between two resisting surfaces. It possesses many fibres of great strength passing on the one hand into the bone of the alveolus of the jaw, and on the other into the *crusta petrosa of the tooth*.

This layer of connective tissue is merely a modification of the periosteum, and a layer of osteoblasts covers the surface of the bone in this region, and another the *crusta petrosa* of the tooth. The transverse uniting fibres are similar in nature to Sharpey's fibres. In the jaw they stop short at any Haversian system they encounter, which will be readily understood when the formation of a Haversian system is remembered.

The alveolar membrane is continuous above with the sub-epithelial connective tissue of the gum. The epithelium (stratified squamous) of the gum (*d*) abuts against the tooth at the junction of the crown and fang, where it terminates. Before terminating, however, it turns downwards a little way along the side of the tooth, so that the blunt end of a needle can be readily inserted a certain short distance between the gum and the tooth, in the living subject, without drawing blood.

The alveolar membrane is continuous below—on the one hand with the pulp of the tooth (*h*), and on the other with the contents of the dental canal in the jaw below the alveolus.

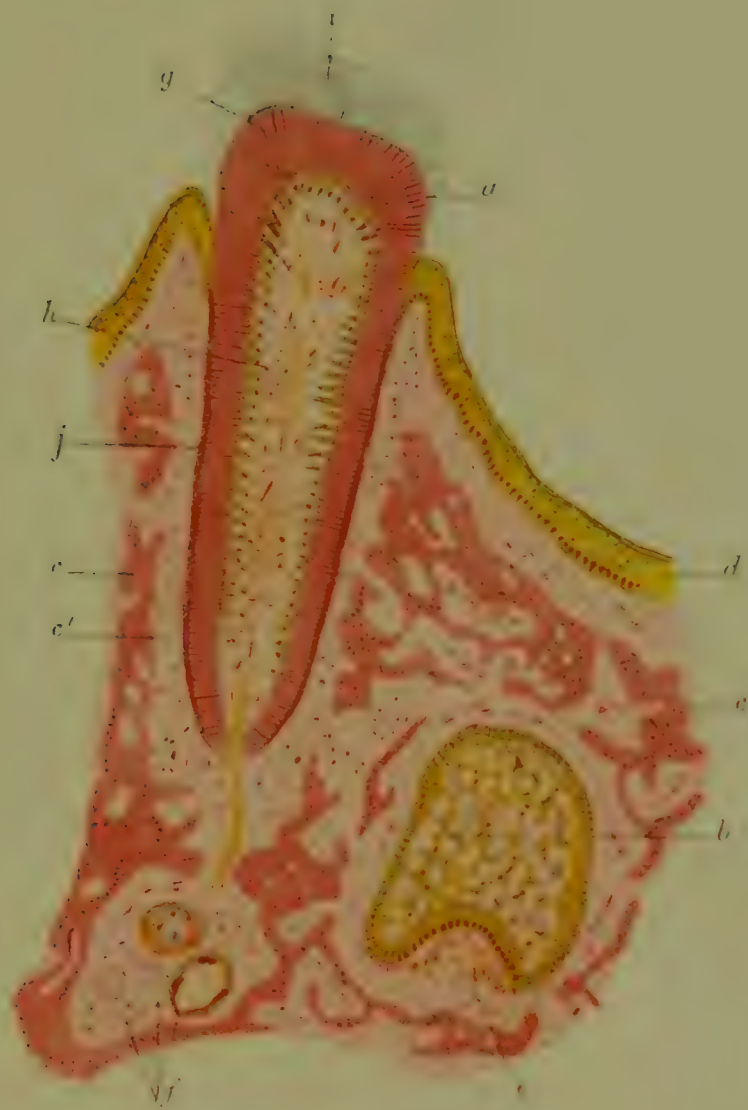
The pulp cavity contains delicate connective tissue supporting blood-vessels and nerve fibres. The cells of the connective tissue at its periphery are specially modified to form the *odontoblasts*, which are continuous with the nerve fibrils. An

FIG. 103.

S. JAW OF KITTEN, SHOWING MILK AND DEVELOPING PERMANENT TEETH, STAINED WITH Picro-Carmine $\times 20$.

- a.*—Milk tooth.
- b.*—Developing permanent tooth, enamel organ stage.
- c.*—Alveolus of jaw.
- c*¹.—Alveolar or periodontal membrane.
- d.*—Epithelium of jaw.
- e.*—Papilla of permanent tooth.
- f.*—Nerve, artery, and vein, in dental canal.
- g.*—Dentine of milk tooth.
- h.*—Pulp of ,,
- i.*—Enamel of ,, (the enamel is not in reality seen in a softened section.)
- j.*—Crusta petrosa of milk tooth.

Fig. 103.



odontoblast is a somewhat pyramidal, nucleated cell, giving off from its base a dentinal fibre, and continuous with a nerve fibril at its apex. The bases of these cells are closely applied to the inner surface of the dentine. The dental canal, running longitudinally in the bone of the jaw beneath the alveoli for the teeth, contains the branches of the dental artery, vein, and nerve (*f*), with connective tissue and lymphatic channels. The artery, vein, and nerve, all send branches upwards, which enter the pulp cavity of each tooth.

DEVELOPMENT OF THE TEETH.

The teeth are developed in two divisions : (1,) the temporary or milk teeth ; (2,) the permanent teeth, the latter replacing the former.

The temporary teeth are developed in connection with primary down-growths of the epithelium of the foetal jaw into the connective tissue beneath. The permanent are developed in connection with secondary growths from the necks of the primary ones.* Each tooth owes its enamel to the epiblast ; its dentine, crusta petrosa, and pulp to the mesoblast.

The milk teeth begin to develop during intra-uterine life. The gum at this time consists of a layer of stratified, squamous epithelium, with connective tissue below it.

The first stage in the development of the tooth consists in a thickening of the epithelium along a line extending the whole length of the alveolus of the jaw—a line which is therefore somewhat horse-shoe shaped. The thickening above, *i.e.*, that seen from the mouth, is termed the *dental ridge* ; that encroaching on the connective tissue below, the *common enamel germ*.† These two projections, above and below respectively, are seen in *Fig. 105, 1*. It will be understood that the section

* Or from the common enamel germ in close proximity to the neck. It should be noted, however, that the three permanent molars are developed independently from a backward prolongation of the common enamel germ, unconnected (in vertical transverse section) with the epithelium of the gum.

† The first down-growth of epithelium becomes divided into two, of which the outer has been named the *labio-dental strand*, and the inner, the *dental lamina*, or *common dental germ*. It is the latter from which the enamel of the teeth is developed, and which is here termed the common enamel germ.

from which the figure is taken is vertical, and transverse to the line of thickening. It is of the greatest importance to realise distinctly the position of this line. It might be represented by supposing the points of eruption of the teeth along the edge of the alveolus, united by a line passing through each of them.

Let us consider for a moment the nature of the epithelium in this region. It consists of a stratum corneum at the surface, which, however, is not always well marked, and a stratum Malpighii beneath it; the latter consisting of more or less polygonal cells, the lowest of which are smaller and more cubical or columnar, and constitute the germinal layer. The common enamel germ consists of a down-growth of both these kinds of cells—namely, the ordinary cells of the Malpighian stratum, and the layer of cubical germinal cells investing the others below.

The second stage in the development of the tooth is shown in *Fig. 105, 2*, and consists in the formation of the *special enamel germ*. This consists of a renewed down-growth of epithelium in the form of a series of flask-shaped bodies from the common enamel germ. These flask-shaped down-growths occur only, however, where the teeth are to be developed. This down-growth is therefore an interrupted, and not a continuous one, as contrasted with that of the common enamel germ. As shown in the figure, each special enamel germ consists of two kinds of cells—in the centre, the ordinary polygonal ones of the stratum Malpighii; and surrounding them, the cubical cells, continuous with the germinal layer. Each enamel germ possesses a body and a more or less constricted neck, from the latter of which a small projection may be seen—the rudiment of the permanent tooth (*e*). The fibrous tissue surrounding the special enamel germ tends to become arranged in a laminated manner conformable to its surface. This laminated tissue, more marked in subsequent stages, is called the *sac* of the developing tooth.

The third stage in the development of the tooth is shown at *Fig. 105, 3*, in which the commencement of a connective tissue *papilla*, and a corresponding invagination of the enamel germ in its lower part, is seen. With the exception of being invaginated from below, however, the enamel germ remains in much the same condition as in the previous stage.

The *fourth stage* is characterised by a further development of the connective tissue papilla (the future pulp) with a correspondingly increased invagination of the flask (*Fig. 105, 4*). But the central cells of the invaginated flask have now undergone a certain change of a degenerative type. They have become branched, mucoid cells, forming a more or less delicate network by the anastomosis of their branches. This stage is shown also in *Fig. 106*; though there the developing tooth is a permanent and not a temporary one. The structure of the *enamel organ*, as it is now called, is the same in either case. It should be especially noted that it is only the central cells which thus degenerate, with the result that between the central degenerated cells and the peripheral cubical cells (continuous above with the germinal layer), we have a narrow layer left of undifferentiated epithelium. Starting from the centre of the enamel organ and passing to the periphery, we thus have at this stage, three layers of cells—degenerated, undifferentiated, and cubical (or columnar). But a section taken through the entire thickness of the enamel organ at any point will show five: (1,) cubical; (2,) undifferentiated; (3,) degenerated; (4,) undifferentiated; (5,) cubical.

The *papilla* is composed of embryonic tissue, in which the fibrous or formed element is scanty, and the cellular predominates. In fact it consists almost entirely of small, round, or oval cells, with large nuclei and little peri-nuclear protoplasm.

The *fifth stage* is represented in *Fig. 107*. It is associated with the commencement of the formation of the enamel and dentine.

The inner layer of cubical cells of the enamel organ, *i.e.*, those immediately surrounding the connective tissue papilla, is the functionally active part of the enamel organ. These cells increase in length till they form a very regular layer of palisades, the nuclei remaining in their external part (*b*). The rest of the organ gradually atrophies, and remains as a thin cap to the tooth (Nasmyth's membrane) for a short time, but finally disappears. The figure shows the increase in the length of the inner cubical cells to form what are now termed *enamel cells*, and the commencing atrophy of the rest of the organ.

But when this stage has been reached in connection with the enamel organ, the connective tissue papilla has also become

specially differentiated. Its peripheral cells assume the characters of odontoblasts, and commence to secrete dentine. The figure shows a layer of enamel (*c*) formed on the inner surface of the layer of enamel cells, and a layer of dentine (*d*) on the outer surface of the layer of odontoblasts. The necessary result of this mode of production of enamel and dentine, is that, as it proceeds, the two layers of cells producing them become more and more widely separated from each other.

The further development of the tooth is merely a continuation of these processes of enamel and dentine formation. As soon as the enamel cap is formed, the cells producing it, together with the rest of the enamel organ, are ready to be discarded as useless. The odontoblasts, on the other hand, persist as a lining to the pulp cavity, where they constitute the peripheral terminations of the dental nerve.

The processes of formation of enamel and dentine require a little further reference. The manner of production of enamel by the enamel cells is somewhat remarkable. If an individual cell is considered, it may be said to consist in the calcification of its inner end, accompanied with a corresponding growth in the length of the rest of the cell. This is shown somewhat diagrammatically at (*f*) in *Fig. 108*. It will be seen that the calcification does not involve the end of the cell through its whole thickness at the same time, but commences at the periphery and extends inwards. The diagram shows the cell withdrawn from its calcified end, into which it will be seen it has fitted as into a kind of socket. In the same figure, a portion of the section from which *Fig. 107* was taken, is shown under a higher power.

Examine the following specimens :—

(I.) *L. Section of unsoftened tooth, human.* (*Fig. 104.*)

In an unsoftened tooth it is impossible to examine anything but the dentine, crusta petrosa, and enamel, as the soft parts are destroyed in the process of preparation.

With the low power, identify the different parts of the tooth : the enamel (*A*), the dentine (*B*), and the crusta petrosa. Note the striation of the dentine, due to the presence of the radiating dentinal tubules (*c*). The crusta petrosa may be recognised as a granular-looking covering to the fang, which narrows as it approaches the neck. Observe the absence of Haversian canals in it, which would appear in this specimen black. Look at the

enamel cap, and note the waviness of the outlines of the prisms (*a*) forming it. The enamel also becomes thinner as the neck of the tooth is approached. At the periphery of the dentine, both in the crown and fang, look for a dark, irregular line indicating the area of interglobular spaces (*b*). In the dentine itself look for what have been termed *incremental* lines—lines parallel with the long axis of the pulp cavity in the fang, and arching over its upper extremity in the crown. These indicate successive stages of calcification of the dentine, and will be better seen in softened sections of developing tooth.

Find the junction between the enamel and dentine, and put on the high power. Examine the wavy prisms of the former (*A*), cut longitudinally. Note the transverse markings upon them. Study the interglobular spaces (*b*); they vary in size, some being large with well-defined outlines, others smaller, and some appearing merely as black granules. Next study the dentinal tubules (*c*). The main tubules are most distinctly seen in the inner part of the dentine, but the branching and anastomosis of the branches are better seen in the outer part. Observe that the branches arise dichotomously, and that when these primary branches give rise to secondary ones, division proceeds in the same way. Examine the junction between the crusta petrosa and the dentine, noting the presence, as before, of interglobular spaces at the edge of the latter. In the crusta petrosa, bone corpuscles, or rather lacunæ, are to be seen, with canaliculi spreading out from them to anastomose with those of neighbouring lacunæ. There are no Haversian systems. The crusta petrosa, therefore, like the enamel and dentine, is extravascular.

(2.) *S. Unsoftened tooth, human, to show enamel prisms and dentinal tubules, cut transversely.* (Fig. 104, C, D.)

A vertical section (of one of the teeth with a pointed crown, and undivided fang), which passes on one side of the pulp cavity, will usually show both these parts in transverse section.

With the high power, study the transverse prisms and tubules. The dentinal tubules are indicated by small round circles, giving a dotted appearance to the dentine (*D*); the enamel prisms, larger and hexagonal, give the appearance of a very regular mosaic (*C*).

(3.) *V.S. Softened tooth in jaw of cat, stained with picrocarmine or hæmatoxylin. F. or B.* (Fig. 103.)

FIG. 104.

V.S. ENAMEL AND DENTINE, UNSOFTENED TOOTH (HUMAN) $\times 200$.

- A.—Enamel.
- B.—Dentine.
- C.—T.S. Enamel prisms.
- D.—T.S. Dentinal tubules.
 - a.*—L.S. Enamel prisms.
 - b.*—Inter-globular spaces.
 - c.*—L.S. Dentinal tubules.

FIG. 105.

SECTION OF JAW OF FÆTAL MOUSE, SHOWING EARLY STAGES OF DEVELOPING MILK TOOTH.

- a.*—Stratum corneum } Epithelium
- b.*— „ Malpighii } of gum.
- c.*—Germinal layer of stratum Malpighii.
- d.*—Subepithelial connective tissue.
- e.*—Germ of permanent tooth.
 - 1.—Stage of common enamel germ.
 - 2.— „ special „ „
 - 3.— „ enamel organ.
 - 4.— „ „ „

Fig. 104.

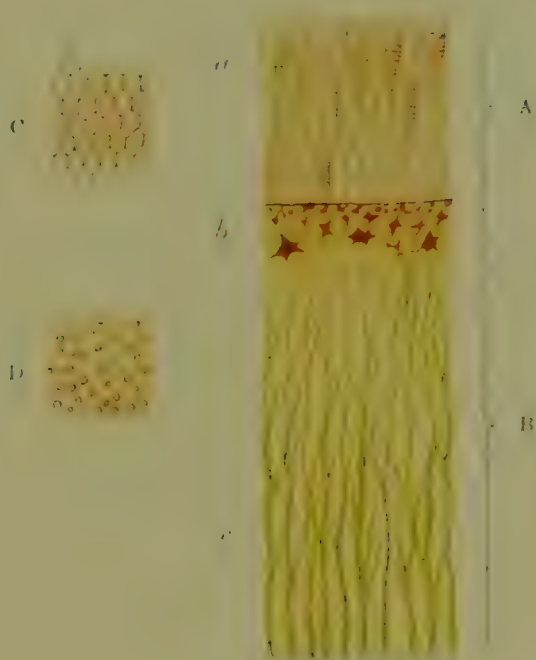
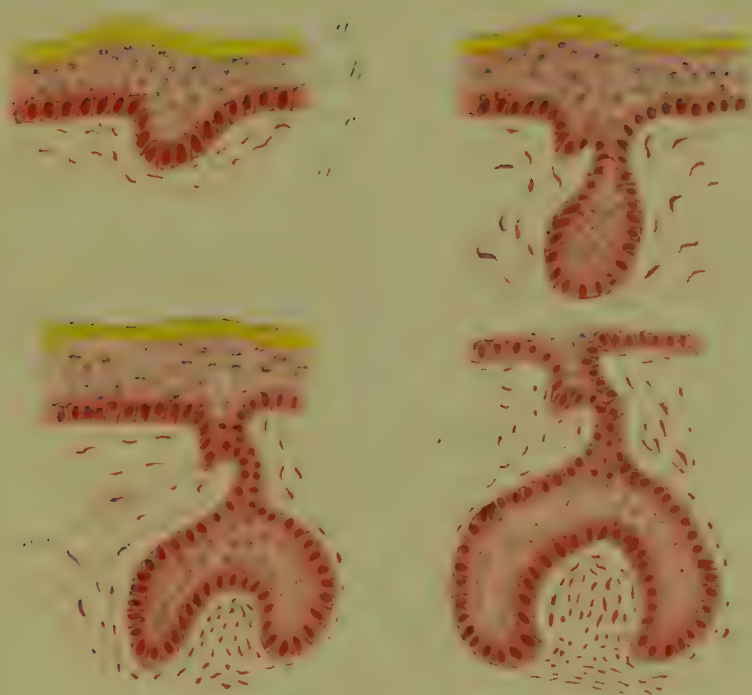


Fig. 105.



To make a preparation of the tooth, together with the soft parts and the bony socket in which it is placed, it is necessary, first of all, to remove all the calcareous matter. The tooth is then cut *in situ* with the piece of jaw belonging to it, and stained and mounted in the ordinary way.

With the naked eye, identify first the different structures which the section shows. First recognise the tooth, then the alveolus of the jaw, with a space in the deeper part of it—the dental canal. This canal, like the pulp cavity of the tooth, is filled with soft tissue, which may or may not fall out during manipulation of the specimen in mounting. Between the tooth and the bone of the jaw, look for the more lightly stained alveolar membrane, and, lastly, recognise the epithelium and sub-epithelial tissue of the gum. In such a section, this mucous membrane often projects as a loose tag on either side, from the neck of the tooth.

Put on the low power, and make a more detailed examination. Arrange the specimen so that the free extremity of the tooth points away from you, when seen under the microscope.

We will suppose that the specimen has been stained with picro-carmin. First examine the tooth itself. The *dentine* (*g*) is stained a deep red with the carmine, and shows the usual striation due to the dentinal tubules. Observe that in the softened section the *enamel* (*i*) is absent. It is entirely dissolved by the acid used in the process. The *crusta petrosa* (*j*) is, however, to be seen as a granular sheath to the fang—a sheath which is thicker in its lower part, and gradually decreases in thickness towards the neck of the tooth. Now examine the contents (*h*) of the pulp cavity, if these have remained *in situ*. Under this power, the pulp, which is lightly stained, presents a dotted appearance, due to the nuclei of its cells, which are stained with carmine. Here and there, nuclei arranged in linear series may be seen, indicating the nerve fibres upon which they occur, and here and there capillary blood-vessels with their yellow contents may be made out. At the periphery of the pulp a more regular arrangement of larger cells than those of its main substance can be distinguished. The characters of these cells, *odontoblasts*, can be better seen in a developing tooth and under a higher power; at present it will be enough to note that they are somewhat columnar in shape, their broad ends directed outwards, and that they are arranged side by side in the

manner of a palisade. Now look at the bone of the jaw stained even more deeply than the dentine with the carmine. Observe the Haversian canals and spaces, with their contents, stained a lighter pink. The *alveolar membrane* (*c*¹) should next be examined. It also is stained much less deeply than either the dentine or the bone between which it lies. Even under this power it is possible to make out the transverse direction of the strong fibres in it which unite the bone to the crista petrosa of the tooth. Observe the frequent sections of capillary blood-vessels in the membrane. Notice immediately in contact with the bone on the one side of it, and with the crista petrosa on the other, a layer of cells—the osteoblasts—the layer characteristic in other situations of the deep division of the periosteum. Trace the alveolar membrane upwards till the highest point of the bone of the jaw is reached, and mark its continuity with the sub-epithelial connective tissue in this region. Trace it downwards to the bottom of the fang, and note its relations here. If the section has so passed through the fang that the opening in its lower end is shown, the continuity of the alveolar membrane with the contents of the pulp cavity will be apparent. Also, if the section has so passed through the bone that the opening from the dental canal towards the tooth is shown, its continuity with the connective tissue lining this canal can easily be demonstrated. But it must be borne in mind that a very little divergence of the knife will admit of both of these openings being missed, in which case the pulp cavity and the dental canal appear as closed spaces with no connection either with the alveolar membrane or each other. We will suppose, however, that the section has been favourable, and that all the connections can be seen. Examine the contents of the dental canal—the *dental artery* with its comparatively thick wall, and the *vein* with its thin one—the *dental nerve*—all cut in transverse section, and supported with delicate connective tissue. Look for indications of branches cut longitudinally from one or more of these passing upwards into the pulp cavity of the tooth.

Finally, observe the epithelium of the gum (*d*) stained yellow in contrast to the pink of the fibrous tissue beneath it. Notice the manner in which it dips down for a short distance along the side of the tooth before it terminates in the region of the neck.

Under the high power, all the parts above referred to should be re-examined ; only those, however, the structure of which is peculiar to the situation, need be referred to again here. Examine the pulp especially for the odontoblasts at its periphery. Trace, if possible, a process from the broad end of one of them into a dentinal tubule. Examine the alveolar membrane again for Sharpey's fibres, tracing them on the one hand into the bone of the jaw, and on the other into the crista petrosa of the tooth. This is one of the best situations in the body in which to identify these fibres. It will be observed that, after entering the bone, they stop short suddenly at the edge of any Haversian system encountered.

(4.) *V. S. Head of fetal mouse, stained with borax-carmine.*
B. (Fig. 105.)

It is advisable to cut all sections of developing tooth in paraffin rather than in gum, as the parts are liable to separate if the sections are floated in water. For the same reason a fixative should be employed. The head of a foetal mouse or other small mammal is especially suitable for the study of the earlier stages of developing tooth. The head of the mouse should be about the size of a large pea, and the section should pass vertically and transversely through both the upper and lower jaws. In favourable specimens, four developing teeth are to be seen—two in the upper jaw, and two in the lower. A considerable number of specimens should be mounted, so as to get sections of teeth in different planes, showing the four stages in *Fig. 105*. Examine such a section with the low power. First of all notice other structures present—the olfactory membrane of the nose, and its transition to respiratory epithelium in the lower part of the nasal cavity. The bone of the upper and lower jaw developing in membrane is very beautifully seen in this specimen; the calcified white fibres standing out as a brightly refractile network. Look particularly at this calcifying membrane of the lower jaw on each side. In the midst of it a transverse section of hyaline (Meckel's) cartilage, in the cellular stage, is to be seen. Now look for the cavity of the mouth, lined with stratified, squamous epithelium. The floor is formed by the transverse section of the tongue. Look for sections of developing teeth, one on each side, above and below. Under this power they can be seen as downgrowths from the epithelial lining into the connective tissue

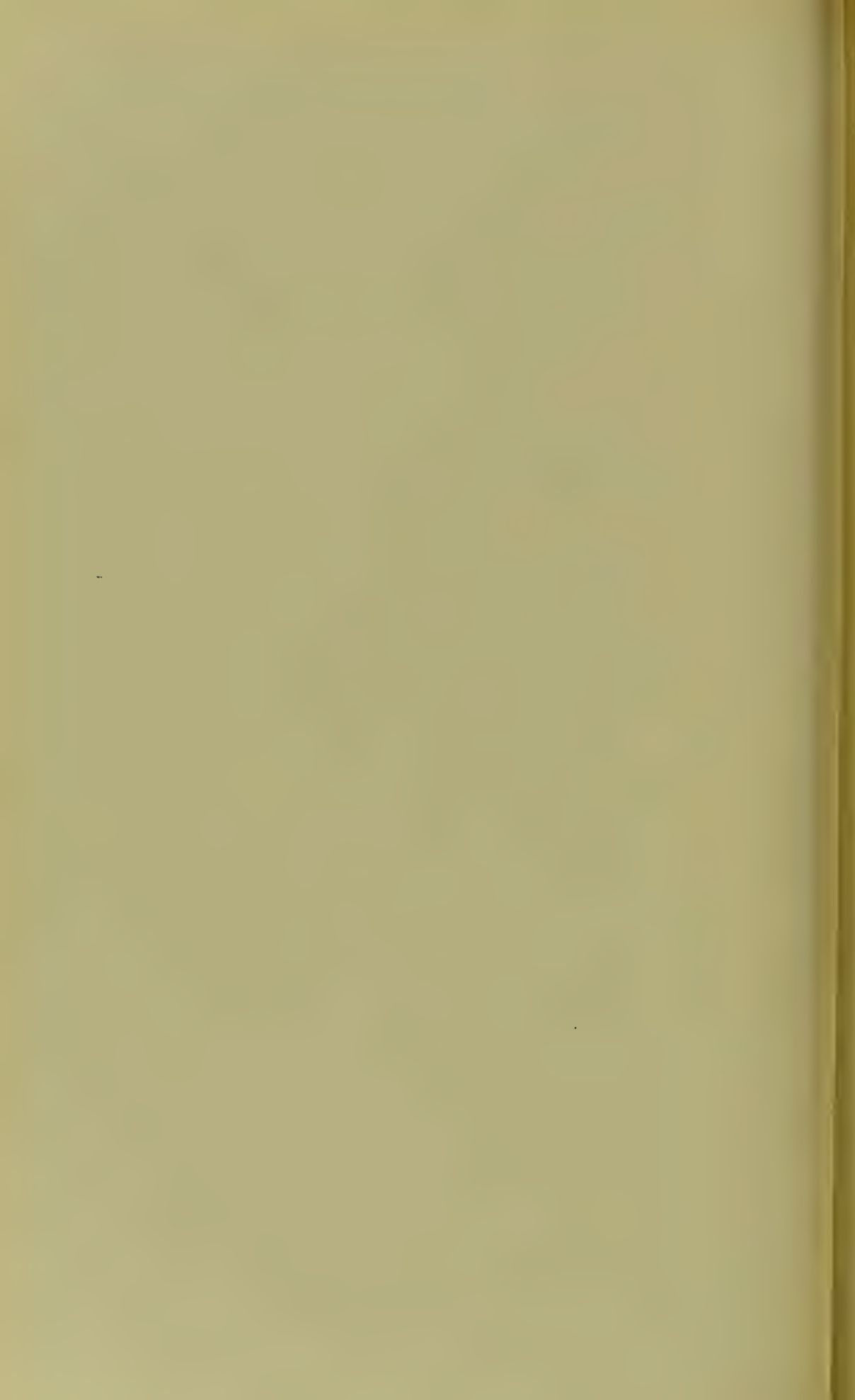
FIG. 106

S. JAW OF KITTEN, SHOWING MILK AND DEVELOPING PERMANENT TOOTH, STAINED WITH HÆMATOXYLIN $\times 40$.

- a.*—Epithelium of gum.
- b.*—Bone of alveolus of jaw.
- c.*—Milk tooth.
- d.*—Permanent tooth, enamel organ stage.
- e.*—Odontoblasts lining pulp cavity of milk tooth.
- f.*—Crusta petrosa of milk tooth.

Fig. 106.





below. Select one of the teeth, and put on the high power. Note particularly the cubical or columnar character of the cells at the periphery of the enamel germ, and trace them into the germinal layer of the stratum Malpighii of the epithelium of the mouth. If development has gone far enough, note the mucoid tissue in the centre of the enamel organ, and the papilla of young connective tissue causing the invagination below. Observe the neck of the enamel organ, and distinguish the projection of epithelium constituting the germ of the permanent tooth (*c*).

Examine also, with the high power, the developing bone of the jaws, and the olfactory and respiratory mucous membrane. In some cases, teeth in a more advanced stage of development may also be well seen.

(5.) *V. S. Softened jaw of young kitten, stained with borax-carmine. B.*

This section is suitable for the study of both milk and permanent teeth in various stages of development. Every stage is well seen, though here it is the permanent teeth which are seen in the early stages, the previous specimen being more satisfactory for studying the commencement of the milk teeth. Several pieces of the jaw, preferably the lower, should be prepared, and after cutting, sections should be selected for mounting, showing the different stages. A very little familiarity with the specimens enables one to pick out the various representative stages with the naked eye.

Figs. 106, 107, and 108, are from the jaw of a kitten, and the student's attention is directed to the following points in connection with them. Fig. 106 shows to the right hand side a portion of one of the milk teeth which has already cut the gum. At (e) are shown the odontoblasts lining the pulp cavity; (c) is the dentine, and (f) the crusta petrosa; (d) represents one of the permanent teeth in the enamel organ stage. Its connection with the surface epithelium is also shown, but it is unusual to have such a perfect demonstration in any one section. Notice that the peripheral cells of the papilla have already begun to assume the characters of odontoblasts. Note the position of the enamel organ of the permanent tooth; it is imbedded to a large extent in the bone of the jaw. This is often more completely the case, as is shown in Fig. 103, where the developing permanent tooth (b) is separated by bone from the temporary one which it is to supplant. As it grows it absorbs the bone between

it and the alveolus of the milk tooth (*a*). It then commences to absorb the lower part of its predecessor, which it ultimately pushes out from below.

Fig. 107 shows the earlier stage of enamel and dentine formation under a low power. In such a section the enamel cells (*b*) are well seen, and the odontoblasts at the periphery of the pulp (*e*). Note the fibrous sac of the tooth (*g*) continuous below with the tissue of the papilla. The enamel cap is stained a very deep carmine, the dentine a lighter carmine.

Fig. 108 shows part of *Fig. 107*, under the high power, the arrow (*f*) in the latter pointing to the part shown. Observe in it the layers of the enamel organ, especially contrasting the length of the enamel cells (*b*) with that of the cubical cells (*h*), with which they are continuous below. Study, under this power, the enamel cells, noting the position of their nuclei, and the denticulate margin they present where they have been torn away from the enamel. This margin owes its character to the manner of calcification of the cells, as described above. Note the corresponding margin of the deeply stained layer of enamel; it also is uneven from the same cause. Observe the broader, more lightly stained dentine, and note here incremental lines (*g*) indicating stages in calcification. Note also the shape of the odontoblasts (*e*), and trace the processes from them into the dentine. These can often be well seen when the odontoblasts have been partially torn away.

THE SALIVARY GLANDS.

The ultimate secretory portion of a compound gland may be, as we have seen, either *tubular* or *saccular* in its nature. These glands are sometimes called *racemose*, from their superficial resemblance to a bunch of grapes.

The terminal portions of a racemose gland, whether saccular, as in the case of the salivary glands, or tubular, as in the case of the pancreas, are spoken of as *alveoli*.

Before describing the salivary glands individually, it will be well to mention the characteristics common to them all. They are compound glands, that is to say, they are removed from the surface by a repeatedly subdividing duct, into the ultimate divisions of which, the terminal alveoli open. Each terminal duct ends in a cluster of such alveoli.

The cells lining the alveoli are the secretory cells of the gland. They are large nucleated cells, arranged in a single layer, with a narrow end directed to the centre of the alveolus they line, and a broader one resting on the basement membrane. This membrane consists of a layer of flattened cells—an imperfect layer, formed of modified connective tissue corpuscles, which is continued in a less distinct form upon the ducts. When the cells lining the alveolus are charged with secretion there is no lumen visible, or only a small one; a lumen becomes visible, or more distinct, however, when the secretion has been discharged, and the cells are consequently shrunk. The alveoli are separated from each other by a small amount of connective tissue supporting a capillary network. The divisions of the duct are, for the most part, composed of a tube of columnar nucleated cells, striated in their outer part. Throughout the greater part of the duct these cells are arranged in a single layer, but they become stratified in the larger branches. The smallest ducts, those immediately opening into the clusters of alveoli, have epithelium of a special character. It ceases to be columnar, and becomes more of the nature of simple squamous epithelium, or, as it is sometimes termed, low cubical epithelium.

The whole gland is surrounded by a *capsule* of connective tissue, from which *trabeculae* pass inwards, separating it into lobes; these are called the *interlobar trabeculae*. From them smaller trabeculae are given off, dividing the lobes into lobules, the *interlobular trabeculae*, and from these still smaller subdivisions, which split up the lobules into smaller areas, and end in the connective tissue between the alveoli themselves. The larger ducts and vessels run in these trabeculae, and are named accordingly. Thus we have *interlobar* ducts, *interlobular* ducts, and *intralobular* ducts, in all of which the epithelium is of the columnar variety. The terminal portion of the duct within a lobule, which opens into the alveoli, and is lined with flattened epithelium, is called the *intermediate* duct. The lumen of such a duct is often larger than that of the alveoli with which it is connected.

The three chief salivary glands are the parotid, the submaxillary, and the sublingual. There are smaller glands also contributing their quota to the secretion of the mouth, called from their position the buccal, the labial, the lingual, and the palatine.

The parotid gland is a serous gland; the submaxillary is chiefly mucous; the sublingual is wholly mucous.

They differ from each other principally in the character of the terminal alveoli. Otherwise they are virtually alike. They each possess a fibrous capsule, sending in processes or trabeculæ which split up the gland into smaller and smaller subdivisions, and in which are found the main blood-vessels and ducts.

The **parotid** may be regarded as a typical serous gland. The alveoli are somewhat rounded in section, or polygonal with rounded corners. They are rather smaller than the alveoli of a mucous gland, and are lined with a layer of serous glandular cells. Generally speaking, as contrasted with mucous cells, these are granular, staining deeply with the reagent, and do not readily transmit the light. The nucleus of the cell is round, and is situated at the junction of the middle and outer thirds. There is little or no visible lumen, and the basement membrane is not very much in evidence.

But both the appearance of the cells themselves, and the existence of a lumen, depends greatly upon the stage of functional activity, or the reverse, in which the gland is examined. The gland may be in a state of rest after secretion, or secretion may be ready to take place. When secretion is about to take place, the cells of the alveolus become laden with granules of some substance, which is a precursor of the secretion, and become swollen in consequence, so that no lumen can be perceived. As the granules do not take up the staining reagent as the protoplasm of the cell does, the cells are not so deeply stained. The granules also, to some extent, obscure the nuclei of the cells. But when secretion takes place, the granules are discharged into the lumen of the alveolus; the cells shrink in consequence, rendering that lumen visible, and stain more deeply, or appear to do so from the consolidation of the protoplasm; and the nuclei again become more apparent. The granules first disappear from the outer part of the cells, and the process of disappearance extends towards the inner ends, till only a few granules immediately around the lumen are visible in the exhausted gland. Thus, in the case of a parotid gland, which is neither completely exhausted, nor completely laden with granules, two zones in the cells of the alveoli may usually be made out; an inner, which, from the presence of granules of comparatively non-stainable material, is lightly stained, and an outer, composed of more condensed protoplasm, which is more deeply stained. This is,

however, more easily seen in the case of the pancreas, which is also a serous gland.

The **sublingual** is a wholly mucous gland. The alveoli are larger than those of a serous gland. Their shape, too, is a little different, their outlines tending to be more circular, or to be made up of segments of different circles. They are lined with a layer of mucous cells, and are invested with a distinct basement membrane, in which the flattened nuclei of the cells forming it are usually apparent. Between the basement membrane and the layer of mucous cells are to be seen, here and there, "demilunes" of cells of the serous type. These demilunes may be separated from each other, or may surround the mucous lining completely. The mucous cells themselves are large and conical in shape, their broad bases resting on the basement membrane, or the demilune cells, and their narrower ends projecting into a small lumen—a lumen which is more in evidence than in the case of serous alveoli, but which is still apt to become obliterated in the loaded condition of the mucous cells. These cells, in hardened specimens, are usually not very granular in appearance; they do not stain deeply, and they readily transmit the light. When they are charged with secretion their nuclei are usually not round, but more or less flattened, and are found quite at the base of the cells. They may be placed either in the centre of the base, or they may be in one corner of it. The demilune cells are found between the mucous cells and the basement membrane. They vary very much in size. Their external outline corresponds with that of the basement membrane on which they are placed, and their internal surfaces are applied to the bases of the mucous cells. They are often triangular, the longest side of the triangle being directed outwards, the opposite angle projecting inwards, between the divergent bases of two mucous cells. They are granular, deeply stained, transmit the light poorly, and have round nuclei.

But, as in the case of the parotid gland, the appearances presented by the alveoli differ according to whether secretion has already taken place, or is about to do so. The above description is, perhaps, rather applicable to the loaded state of the gland cells preceding secretion. At this stage the interstices of the protoplasm of the cells are loaded with granules of the precursor of mucin, which do not stain with ordinary reagents, the protoplasm remaining merely as a delicate network with widely dis-

FIG. 107.

T.S. DEVELOPING TOOTH OF KITTEN, SHOWING FORMATION OF ENAMEL AND DENTINE, STAINED WITH PICO-CARMINE $\times 100$.

- a.*—Outer layer of enamel organ.
- b.*—Inner " " " (enamel cells).
- c.*—Enamel.
- d.*—Dentine.
- e.*—Pulp of tooth, with odontoblasts at periphery.
- f.*—Part from which *Fig.* 108 is taken.
- g.*—Fibrous sac.

FIG. 108.

S. DEVELOPING TOOTH OF KITTEN, STAINED WITH PICO-CARMINE $\times 350$.

- a.*—Atrophying layers of enamel organ.
- b.*—Enamel cells.
- c.*—Enamel.
- d.*—Dentine.
- e.*—Odontoblasts.
- f.*—Enamel cell, under a higher power, showing calcified portion (enamel), detached from uncalcified.
- g.*—Points to position of incremental line running parallel with the surface of the dentine (the line has been omitted inadvertently).
- h.*—Cubical outer cells of enamel organ, continuous with enamel cells (*b*) below.



Fig. 108.



tended meshes. When the mucinogen granules have been discharged into the lumen of the alveolus, the cells shrink, the protoplasmic network again becomes closer, and thus appears to stain more deeply, the nuclei become round, and more centrally placed, and the lumen of the alveolus is greatly increased in size. As in the case of the parotid gland, the outer part of the cell is the first to become free from the granules of mucinogen, and assume the ordinary protoplasmic appearance, but as in the case of the parotid, so also, it may be said, of the sublingual, that the distinction of the cells into two zones, an outer more deeply stained, and an inner less deeply from the presence of granules of the precursor of the secretion—granules which do not stain with the ordinary reagents—is not nearly so easily made out as in the case of the pancreas. In fact, in an ordinary specimen of a mucous gland, it is not possible to make out two zones in the cells. With regard to the demilunes, it may be noted that these are not present in all salivary mucous glands. They seem to be absent from the mucous alveoli of the sublingual gland of the guinea pig, and the submaxillary gland of the mole. In the submaxillary gland of the dog the demilunes occur at intervals between the basement membrane and the mucous cells; in the submaxillary of the cat they form almost a complete investment to the alveoli. It should be noted, too, that mucous glands in different situations in the same animal often show differences with regard to these cells. Thus, in the sheep there are no demilunes in the mucous glands of the trachea and œsophagus; whereas in those found in the mucous membrane of the epiglottis, the parietal demilune cells are very large.

In both serous and mucous glands it must be noted that the granules spoken of as the precursors of the secretion, do not in hardened preparations give a granular appearance. It is the protoplasm proper of the cell which is granular, and it is the appearance in the granular protoplasm of "granules" of comparatively unstainable material which renders the cell, in whole or in part, less deeply stained.

In connection, therefore, with the loaded and exhausted condition of the cells of these glands, either serous or mucous, we have to note that in the loaded condition of the cell it is larger, and stains less deeply; when the exhausted condition is ensuing, the granules of unstainable material are poured into the lumen of the alveolus from the central ends of the cells, their place

being taken by the granules in the peripheral. Hence the first part of the cell to become clear of the precursor of secretion is the peripheral part; the last part to contain it the central. Thus, the peripheral again stains deeply before the central part does. When exhausted the cells are small, and the lumen of the alveolus more distinct. But the glands are not often found in a really exhausted condition. To bring this about prolonged faradisation of the nerve seems to be required. The following are some of the chief points of difference between serous and mucous alveoli:—

SEROUS GLAND.	MUCOUS GLAND.
Alveoli comparatively small.	Alveoli comparatively large.
Basement membrane not prominent.	Basement membrane prominent.
No demilunes.	Demilunes may be present.
Cells granular and deeply stained.	Cells not so granular and lightly stained.
Cells do not readily transmit light.	Cells readily transmit light.
Nuclei round and placed at junction of outer and middle third of cells.	Nuclei usually flattened and placed at base of cells.

These columns show the differences between the alveoli in detail, but they are not to be relied upon individually as a means of differential diagnosis. They should be taken collectively. Thus, in the alveoli of some mucous glands there are no demilunes; and an exhausted mucous cell may be as granular as a loaded serous one. The main point is to get to recognise the general appearance of these two kinds of alveoli, and to employ the above table as a confirmatory test.

A mucous gland may generally be recognised by the size of the alveoli; the dark contour caused by the prominent basement membrane with, it may be, demilune cells; the transparency of the cells, *i.e.*, the readiness with which they transmit light. This last is an exceedingly important characteristic, and gives to the section under either power a *primâ facie* appearance, which often enables one to come to an immediate conclusion as to the nature of the gland. The shape and position of the nuclei should be determined. A serous gland can generally be recognised by the comparative smallness of its alveoli, the absence of a dark contour, and the obstruction they afford to transmitted light. Next, by the shape and position of the nuclei. The

absence of demilunes should also be noted, but the student should be on his guard against passing these over when they are in reality present. In some mucous glands they are so narrow in section as to cause little more than a thickening, here and there, of the dark contour of the basement membrane.

The **submaxillary** in man is a mixed gland, for the most part mucous, but to some extent serous. That is to say, the majority of the terminal ducts open into clusters of mucous alveoli, but some into clusters of serous alveoli. A section of the submaxillary gland is, therefore, favourable for the purpose of comparing the different alveoli. Of the smaller glands of the mouth, those opening into the valla of the circumvallate papillæ are serous; the rest are mucous, and without demilunes.

Study the salivary glands in the following sections, which should have been cut in paraffin. If cut in gum they are very liable to break up when they get into water, and almost invariably do so, unless they have been cut so thick that they are of little use for inspection with the high power.

Pieces of the glands cut small may be hardened in alcohol, osmic acid, picric acid, corrosive sublimate, or Flemming's solution, and then stained in bulk with borax-carmin.

But it is important to note that the granules produced in the cells of serous glands are dissolved by alcohol, chromic acid, and other usual hardening reagents, and so are not seen in most permanent preparations. In mucous glands, too, the granules of mucinogen readily swell up under the influence of reagents, and become transformed, while still within the cells, into transparent mucin. As we shall see in considering the pancreas, the granules in the cells of that gland are not so readily broken down, and may be fixed and stained with osmic acid.

The granules may be advantageously studied in any of these glands, by teasing out a small portion in normal saline after removal from a recently killed animal.

(1.) *S. Submaxillary gland of rabbit, stained with hæmatoxylin.*
B. For serous alveoli. (Fig. 110.)

Examine first with the low power. Observe the main mass of the gland stained deeply, broken up into subdivisions of various shapes, cuneiform, triangular, etc., by processes of connective tissue passing inwards from the more lightly stained capsule. Note the branches of ducts and blood-vessels running in these more lightly stained trabeculæ between the lobules. Observe

the ultimate alveoli of the lobules, small, deeply stained, somewhat round bodies. Even under this power the nuclei in the cells lining them, and in those forming the ducts, may be made out. Put on the high power, and examine the alveoli and ducts. Examine first the alveoli, noting their granularity and depth of staining. Observe that they appear to be solid blocks of cells. It is not easy to find an alveolus with a distinct lumen. If the gland has been taken previous to secretion (B), *i.e.*, when the cells are in a loaded condition, none of the alveoli show any lumen. It must always be remembered, however, that in either a mucous or serous gland, an alveolus may be so cut that the section passes to one side of the lumen. Notice the absence of a dark outline to the alveoli, and the smallness of the amount of connective tissue between them. It is so small as to look merely like a light line separating neighbouring alveoli from each other. Two zones may or may not be distinguishable in the gland cells. Usually, however, the outer part of the cell does appear a little the more deeply stained. Note the round nuclei stained deeply, and placed in the outer third of the cell. These nuclei are much larger in proportion to the cells than is the case with the corresponding nuclei in mucous alveoli. Examine the ducts cut, some longitudinally, and some transversely. The larger ones are found in the interlobular septa, and in these the epithelium is frequently stratified. Find a medium sized one, either in the septa or among the alveoli, and study its single layer of tall columnar cells, striated in the outer part, and with an oval nucleus placed nearer to the central end of the cell. The long axis of the nucleus is in that of the cell. The intermediate ducts (*b*) are not easily seen; they appear as two parallel lines (when cut in longitudinal section) of flattened nucleated cells, not unlike blood capillaries at first sight. But the nuclei in their walls are placed closer together as the cells forming them are not so flattened.

(2.) *S. Sublingual gland of guinea pig, stained with borax-carminé. B.*

Under the low power observe the general arrangement of the gland as before, and then contrast this with the preceding specimen. Even under this power certain differences are at once perceptible. In the first place, the whole section transmits the light very much more readily, that is, it is very much more transparent. Notice next that the alveoli are considerably larger, and much less deeply stained. They have the appearance, too, of

possessing a deeply stained border, though no demilunes are to be seen. Look for the nuclei of the mucous cells, and observe that under this power the cells appear to be without them, at least, as far as the main part of them is concerned. Notice how distinctly the sections of the ducts stand out from the alveolar portion of the gland over the field generally. The nuclei of the cells lining them can be distinctly made out. Now put on the high power, and study an alveolus. Observe that in the sublingual of the guinea pig we have to deal with mucous alveoli without demilunes, all the cells being of the mucous type. Look at the outline of the alveolus, and note how the dark contour seen under the low power is produced. A well defined basement membrane of flattened connective tissue cells, whose nuclei are now plainly visible here and there, is mainly responsible for it, but the nuclei of the mucous cells themselves, by their shape and position, have something to do with it. They are small compared with the size of the cells, flattened, and in most cases pressed up against the base of the cell. Sometimes they are to be seen in the corner of the base of the cell, when they are apt to assume a somewhat triangular shape. The nuclei, of course, are stained deeply with the carmine. Now look at the protoplasm of the mucous cells. There is a certain amount of granularity about it, but it does not cause the cell to stain deeply, nor does it interfere with the transmission of light. Thus, it may be noted again here, that mucous glands, unless specially exhausted by faradisation of their nerves, are usually sufficiently charged with the granules, which are the precursors of secretion, to render the cells of their alveoli transparent after they have been subjected to the action of reagents. Between the transparent granules the protoplasm of the distended cell exists as a very delicate network, to which the granularity of the cell is due. It is only after artificial exhaustion that this protoplasmic network becomes sufficiently consolidated to obstruct the transmission of light in the way that a serous cell does normally. The outlines of the individual cells are very distinctly marked, standing out as bright refractile lines. Alveoli showing a distinct lumen are readily found. Now examine the ducts, noting the rodded nature of the outer part of the cells lining them, when these cells are in the form of a single layer of columnar ones. Note the stratified epithelium of the larger ducts. Look amongst the alveoli for an intermediate duct. They are usually easily found

FIG. 109.

S. SUBMAXILLARY GLAND OF DOG, STAINED WITH BORAX-CARMINE $\times 300$.

- a.*—Interlobular duct, ending in,
- b.*—Intermediate duct.
- c.*—Alveolus, showing lumen.
- d.*— „ cut ex-centrally.
- e.*—Connective tissue between alveoli.
- f.*—Parietal cell (demilune).
- g.*—Central mucous cell.
- h.*—Nucleus of basement membrane cell.

FIG. 110.

S. SUBMAXILLARY GLAND OF RABBIT, STAINED WITH HÆMATOXYLIN
 $\times 300$.

- A.—Nerve stimulated $6\frac{1}{2}$ hours.
- B.—Resting gland, *i.e.*, loaded.
 - a.*—Alveoli.
 - b.*—Intermediate duct.
 - c.*—Connective tissue.

Fig. 109.

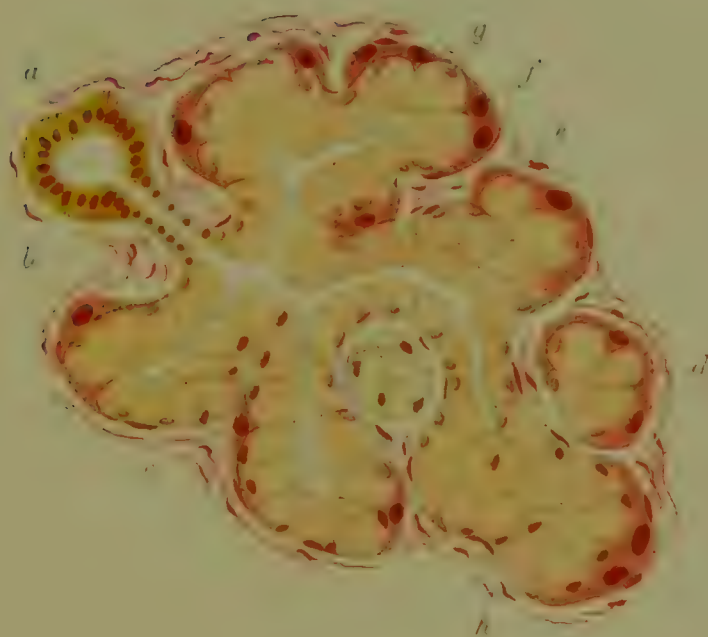
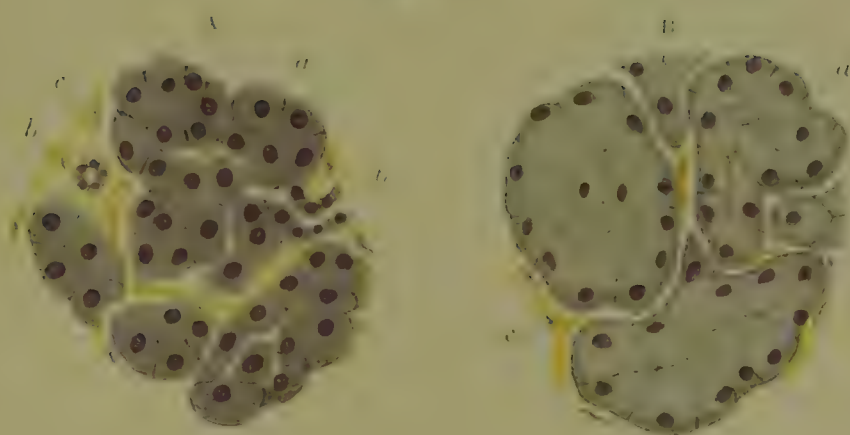


Fig. 110.



in the neighbourhood of an intralobular duct with columnar epithelium, and may often be seen coming off from such a one, and may be traced into the alveoli in which they terminate. They stand out amongst the alveoli on account of their deeper staining, which is due, to some extent, to the nuclei of the cubical or flattened cells lining them.

(3.) *S. Submaxillary gland of dog, stained with borax-carmin.* B. (Fig. 109.)

Under the low power observe the characters of the alveoli as in the last preparation, but note that here they are partially surrounded with deeply stained demilune cells. Put on the high power over a selected group of alveoli. Study the demilunes (*f*). They are usually somewhat semilunar in shape, with a point projecting from some part of the concavity, running inwards a short distance between the bases of two of the mucous cells. Thus the inner margin of a demilune is made up more frequently of two concavities than of one. A nucleus, round or oval, is usually to be seen in the thickest part of the cell. One might compare these cells in shape to a cusp of one of the semilunar valves of the heart, the projecting spur corresponding to the corpus Arantii. Look for a small intralobular duct (*a*) giving off an intermediate branch (*b*).

(4.) *S. Epiglottis of sheep, stained with picro-carmin or borax-carmin.* F. or B.

Under the low power observe the central core of elastic cartilage, the stratified squamous epithelium investing the whole, and, between the two, subepithelial connective tissue, containing clusters of mucous alveoli, whose ducts open upon the surface of the mucous membrane. Select a group of alveoli, and put on the high power. Note the very large size of the granular, deeply stained demilunes, which here completely surround the central mucous cells.

(5.) *S. Human submaxillary gland, stained with hæmatoxylin and methyl-blue (or submaxillary of guinea pig).* B.

This method of double staining, if carried out successfully, yields an exceedingly beautiful contrast between the mucous and serous alveoli. The sections, which may be cut in gum, should be stained with hæmatoxylin, and washed in water in the ordinary way, and then transferred to a solution of methyl-blue for a few minutes. They should then, after rapid washing in water, be mounted in Farrant or Canada balsam. The latter

requires great care to be taken that the methyl-blue does not become entirely removed in the processes of dehydration and clearing.

Under the low power notice how beautifully the clusters of mucous alveoli are picked out with the methyl-blue. In such a specimen the similarity of the clusters to bunches of grapes, in their general arrangement, may often be exceedingly well made out. Put on the high power over a group of mucous alveoli, and contrast the mucous cells here appearing homogeneous, and stained a very bright blue, with the duller granular hæmatoxylin stained demilunes. Move the specimen a little till some of the serous alveoli come into view, and note that the cells present exactly the same appearance, as far as staining and granularity are concerned, as the demilunes of the mucous alveoli.

Notice the sections of the medium sized ducts. The cell protoplasm is a brownish yellow colour ; the nuclei are stained blue. It may be noted that in specimens of the salivary glands stained with hæmatoxylin, this is the usual appearance presented by the columnar epithelium of the ducts. Thus they are distinguished with great ease from the alveoli, in which the protoplasm of the cells stains in the ordinary manner.

APPENDIX TO CHAPTER XI.

METHODS OF PREPARATION.

1. Tongue of cat or dog.—Cut the tongue by vertical transverse sections into several pieces. Harden in Müller, or Müller and spirit; cut in gum, and stain in picro-carmin or hæmatoxylin.

2. Circumvallate papillæ.—Select portions of tongue of man or sheep showing these. Harden and cut as for 1. Stain in picro-carmin or hæmatoxylin.

3. Papilla foliata of rabbit.—Excise the small leaf-like area on either side of the tongue towards its base. Harden in Müller and spirit, cut in gum, stain in picro-carmin or hæmatoxylin, and mount in Farrant or balsam; or stain in bulk in borax-carmin, and cut in paraffin. Care requires to be taken in recognising the direction of the furrows before embedding, so that they may be cut vertically and transversely.

4. Injected tongue of cat, rabbit, or guinea pig. Excise the tongue of a small mammal which has been successfully injected from the aorta with carmin or blue gelatine mass; harden in Müller, or Müller and spirit, cut in gum, and mount in balsam.

5. Jaw of cat for softened tooth.—Kill a cat and remove its lower jaw. Place in chromic and nitric fluid till decalcified. With a sharp scalpel or razor cut the jaw into pieces, by vertical transverse sections, including a tooth in each. Cut in gum, stain in picro-carmin or hæmatoxylin, and mount in Farrant or balsam; or stain in bulk in borax-carmin, and cut in paraffin. The latter method is preferable if the pulp is to be retained in position.

6. Head of foetal mouse for early stages of developing tooth. The head should not require softening; if it does the earliest stages of development of the milk teeth will not be seen. Harden in Müller and spirit, stain in bulk in borax-carmin, and cut in paraffin.

7. Jaw of young kitten (softened) for developing tooth. Obtain a kitten in which the molar teeth have just been cut. Treat the jaw as for 5. Stain in bulk in borax-carmin, and cut in paraffin. Several pieces may be cut at the same time, so as to get all the different stages of development of the permanent teeth; the milk teeth are already developed.

8. The salivary glands.—The glands of any small mammal are suitable, cat, rabbit, guinea pig, rat, dog, etc. (*a*,) For general arrangement of parts under the low power, harden the gland in Müller and spirit, cut in gum (fairly thick sections), stain in hæmatoxylin, and mount in balsam. (*b*,) For

study of details under the high power, more rapid methods of hardening are advantageous, and the tissue should be cut in paraffin, as sections cannot be cut sufficiently thin otherwise. If cut thin in gum they break to pieces as soon as they are floated in water. The following reagents may be employed : (1,) Alcohol. Place small pieces in 75 per cent. alcohol for an hour, and transfer to absolute alcohol for twenty-four hours. Stain in bulk in borax-carmines, and cut in paraffin. (2,) Osmic acid, 1 per cent. solution. Place small pieces, $\frac{1}{8}$ inch cube, in osmic acid for twenty-four hours ; complete in alcohol ; stain and cut as for 1. (3,) Flemming's solution. It acts in a few hours, but the pieces must be small (page 13). Then wash in water for some hours, and transfer to alcohol. Stain in bulk in Kleinenberg's hæmatoxylin, and cut in paraffin. (4,) Corrosive sublimate. Use a half saturated alcoholic solution. Place small pieces in it for an hour or more ; wash the sublimate out with 75 per cent. alcohol for some hours before transferring to ordinary spirit. Stain in bulk in borax-carmines or hæmatoxylin ; cut in paraffin. (5,) Picric acid saturated solution. Wash out the picric acid in 75 per cent. solution alcohol before transferring to spirit. Stain in bulk in borax-carmines or hæmatoxylin, and cut in paraffin.

CHAPTER XII.

*THE ALIMENTARY CANAL (continued).**THE LIVER AND PANCREAS.*

THE ŒSOPHAGUS.

THE Œsophagus or gullet consists of the following layers from within outwards.

- (1,) *The Mucosa*.—(a,) *Epithelium* ; (b,) *Connective Tissue* ;
(c,) *Muscularis Mucosæ*.
- (2,) *The Submucosa*.
- (3,) *The Muscular Coat*.
- (4,) *The Fibrous Investment*.

The *epithelium* lining the **mucosa** of the Œsophagus, like that of the mouth, is epiblastic in origin, and is of the stratified squamous variety. Its deepest cells are small, somewhat columnar in shape, with large nuclei, and correspond with those similarly situated in the epithelium of the mouth and skin. They are, in fact, germinal cells, and by their division replace the cells removed by attrition from the surface. These superficial cells are, for the most part, non-nucleated periplasts, enclosing a homogeneous substance, keratin, into which the original protoplasm of the cell has been converted. Between the superficial flattened squames, of which there are several layers, and the deepest germinal layer, the cells have an intermediate character. Thus, the epithelium is virtually similar to that lining the mouth, except that it is not divisible into two distinct layers, the stratum corneum and the stratum Malpighii.

As in the case of the epithelium of the mouth, the line between it and the subjacent connective tissue, is not a straight one, the

latter being prolonged upwards in the form of fibrous papillæ, between which corresponding downward prolongations of the epithelium fit in. The surface of the epithelium, however, does not follow these downward prolongations, but presents a tolerably uniform appearance.

The layer of epithelium as a whole is, however, thrown into longitudinal folds in such a manner, that in a transverse section of the tube the lumen is nearly obliterated, and exists merely as a radiating fissure. On distension of the œsophagus, these folds disappear in the same manner that the folds of the mucosa of the bladder disappear when it is filled with water.

Between the epithelium and the muscular coat is a broad layer of loose connective tissue, divided in the lower part of the tube, by the presence of the muscularis mucosæ, into two layers, an internal narrow one belonging strictly to the mucosa proper, and an external broader one to the submucosa. Inasmuch, however, as this division is not well marked in the œsophagus, the mucosa and submucosa may be conveniently described together as far as their connective tissue element is concerned, it being noted that in the rest of the alimentary canal the muscularis mucosæ marks off the one from the other very distinctly.

The connective tissue of the **submucosa** is of the loose, areolar variety, and thus allows of considerable movement between the epithelium and the muscular coat. It contains blood-vessels lymphatics, nerves, and mucous glands, whose ducts open into the lumen of the tube. These glands are much more numerous in the œsophagus of the dog and some other animals than in man.

Immediately beneath the epithelium the connective tissue is closer and finer in character, and is raised into small papillæ. The finer blood-vessels and lymphatics contained in these papillæ are connected with the larger vessels in the looser tissue outside the muscularis mucosæ. At a little distance from the epithelium the muscularis mucosæ is represented by separated longitudinal strands of non-striped muscular fibres, except in the upper part of the œsophagus where it is altogether absent. It forms a more complete layer as the stomach is approached, and at the cardiac orifice becomes continuous with the muscularis mucosæ of that viscus.

Outside the muscularis mucosæ is the submucous layer proper,

containing the large vessels and glands. These mucous glands are without demilunes. Their ducts narrow when they reach the epithelium, but are considerably distended as they lie in the submucosa. Small masses of adenoid tissue are to be seen here and there.

The **muscular coat** is arranged in the form of two layers, an internal circular and an external longitudinal. Both layers are composed of striated fibres in the upper third of the tube, and of non-striated in its lower half. The intermediate portion consists of an admixture of the two, and represents a transitional area.

Above, the layers terminate in the inferior constrictor of the pharynx; below, in the muscular coverings, circular, oblique, and longitudinal, of the stomach.

Outside the muscular wall of the Œsophagus, is a **fibrous sheath**, sending septa between the bundles of the external longitudinal muscular coat, of which it may be said to form the perimysium.

Study the structure of the Œsophagus in a transverse section of that of the dog or pig.

(1.) *T. S. Œsophagus of pig, stained with hæmatoxylin and eosin. B. (Fig. 112.)*

Under the low power observe the layer of epithelium, stained much more deeply than the connective tissue outside it. The germinal layer of cells, as in the case of stratified squamous epithelium found elsewhere, has the appearance of being more deeply stained with the reagent, owing to the proximity of the nuclei of the small cells composing it, to each other. In the more lightly stained connective tissue (*b*), between the epithelium and the muscular coat, note especially the clusters of mucous alveoli with ducts cut in various directions; some of them longitudinally as at (*g*), where one is shown opening upon the surface; others, transversely, as at (*h*). Observe the expanded condition of most of these ducts before they reach the epithelial lining; as they traverse the layer of epithelium, their lumen is, however, very much contracted. Look for evidence of the presence of the *muscularis mucosæ* (*i*). This, it must be remembered, is not present in the upper part of the Œsophagus, and, except at the lowest, forms a very incomplete layer. Under this power, if present, it is seen merely as a series of transversely divided strands, embedded in the connective tissue a little outside the epithelium. Two or three fibres of non-striped muscle may

FIG. III.

S. PANCREAS OF DOG, STAINED WITH BORAX-CARMINE \times 350.

- a.*—Intermediate duct.
- b.*—Terminal alveoli.
- c.*—Another intermediate duct.
- d.*—Fibrous tissue septum.
- e.*—Lightly stained central part of cell.
- f.*—Deeply stained peripheral part of cell.
- g.*—Intermediate duct, cut across as it issues from alveolus.

FIG. II2.

T.S. CÆSOPHAGUS OF PIG, STAINED WITH HÆMATOXYLIN AND EOSIN \times 40.

- a.*—Epithelium.
- b.*—Subepithelial connective tissue.
- c.*—Internal circular muscular coat.
- d.*—External longitudinal muscular coat.
- e.*—Fibrous tissue investment.
- f.*—Lumen of cœsophagus.
- g.*—Opening of duct of mucous gland.
- h.*—Group of mucous alveoli ; with duct in centre cut transversely.
- i.*—Isolated strands of muscularis mucosæ.

Fig. 111.

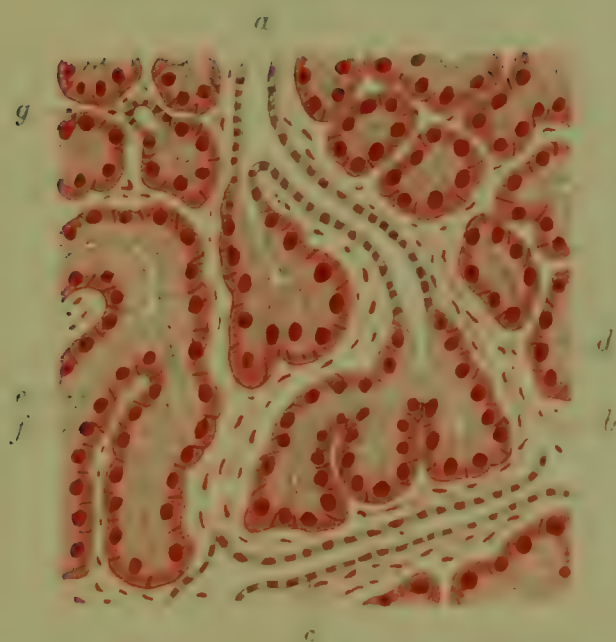
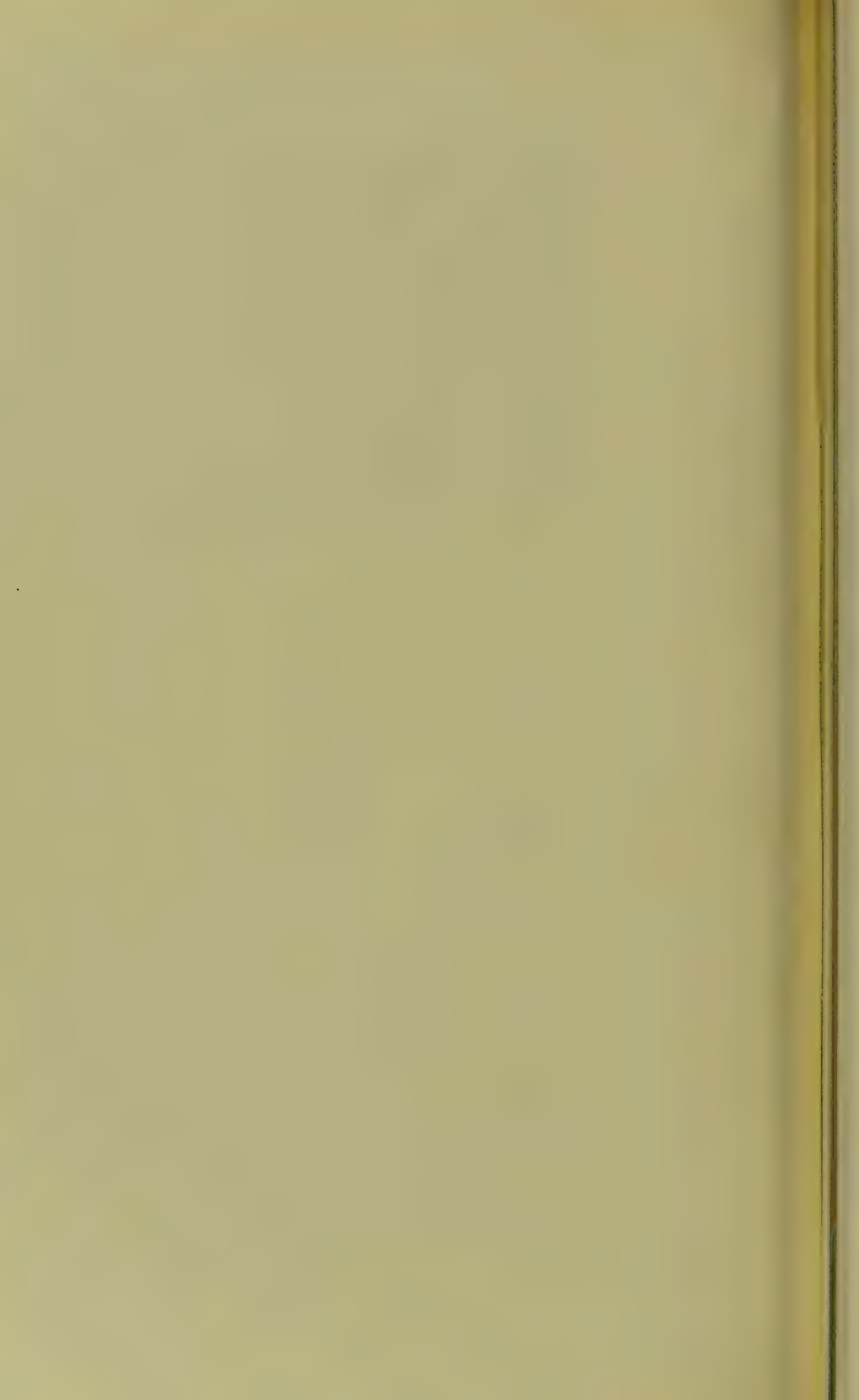


Fig. 112.





go to each strand. These small "blocks," as they appear in transverse section, are stained a reddish colour with eosin, and stand out distinctly from the connective tissue surrounding them.

Outside the submucous coat identify the divisions internal and external of the broad, well marked muscular layer, stained a red colour with the eosin (*c* and *d*). When the section has been taken sufficiently high up to include the constrictors of the pharynx, the definite arrangement in two layers, circular and longitudinal, is to a great extent lost.

Under the high power study the structure of the parts mentioned, in greater detail. Find a duct opening upon the surface of the epithelium (*g*). Trace it outwards into the connective tissue, noting its continuity with the cells of the epithelial lining. The wall of the duct itself, between the epithelium and the glands, consists of a single layer of nucleated, low, cubical cells. Examine the alveoli of the glands (*h*). They are of the mucous variety. The nuclei are towards the bases of the cells, the lumen is often large, and there are no demilunes. Next examine the transverse sections of the fibres of the muscularis mucosæ (*i*). They are stained red with the eosin, and when the section has passed through the nucleus, this is seen in the centre stained blue with the hæmatoxylin. Passing outwards, study the muscular coat proper of the œsophagus, identifying it as composed of striated or non-striated fibres, as the case may be. If striated, the transverse sections of the longitudinally running fibres of the external division will be large, with their nuclei stained with hæmatoxylin, at the periphery of the fibre, *i.e.*, beneath the sarcolemma (as in *d*). If non-striated, they will be small, with a central nucleus when the knife has passed through it. The internal longitudinally cut circular division will also show, by the size of the fibres, whether it is composed of striated or non-striated muscle.

Outside the muscular coat, observe the fibrous sheath (*e*) sending septa between the muscular bundles.

THE STOMACH.

The stomach consists of the following layers from within outwards.

- (1,) *The Mucosa*.—(*a*,) *Epithelium* ; (*b*,) *Connective Tissue* ;
(*c*,) *Muscularis Mucosæ*.

(2,) *The Submucosa.*

(3,) *The Muscular Coat.*

(4,) *The Peritoneal Investment.*

The *epithelium* lining the **mucosa** is hypoblastic in origin. It forms for the most part a number of tubular glands placed side by side, separated by a small epithelial area, constituting the immediate lining of the viscus. The glands are of two kinds, cardiac and pyloric.

The former, as their name implies, are found at the cardiac end of the stomach. They are sometimes simple tubular glands, but are more frequently compound, presenting two or three divisions. They have a mouth, a neck, and a body. The mouth of the gland is short compared with the body, and may represent a fourth in length (or a little more), of the whole tube. It opens on the cavity of the stomach, and is lined by columnar cells, continued as the immediate lining of the organ from the mouth of one gland into those of its neighbours. These cells are mucin forming. The outer half of each is clear and homogeneous; the inner basal half is granular, and contains an oval nucleus. Seen on surface view they present the appearance of an hexagonal mosaic. The cells are tallest where they cover the ridges between the mouths of the glands; shortest in that part of the mouth joining the neck. The neck marks the junction of the mouth or duct with the body or secreting part proper of the gland. This is lined by cells of two kinds, chief or central, and parietal.

The chief cells are columnar in shape, though not so tall as those lining the mouth or duct, with which they are continuous. They are faintly granular, and possess a rounded nucleus placed towards the base. They form a complete layer around a narrow and tortuous lumen; markedly narrow when contrasted with the lumen of the mouth. The parietal cells are oval in shape, much larger than the foregoing, nucleated, and coarsely granular. They are placed outside the others, except in the region of the neck, where they may reach as far as the lumen; but for the most part they are placed between the layer of central cells and the basement membrane, the latter of which tends to be bulged outwards to accommodate them. They do not form a continuous layer. They differ in number and size at different parts of the body of the gland. Thus, below the neck, they are comparatively numerous, smaller, and so arranged that they may abut upon

the lumen of the tube as well as the basement membrane. In this position the long axis of the cell seems to be vertical to the basement membrane. Below, they are less numerous, larger, and are placed entirely outside the layer of chief or central cells. Here, their plane seems to be horizontal to the basement membrane. Owing to their clear outline, their granular appearance, and the fact that they stain deeply with reagents, these cells are much more readily seen than the central ones.

Fig. 113 shows two of these glands, the one simple, and the other compound.

The pyloric glands differ from the cardiac, as follows: Their ducts or mouths are longer and wider; their bodies are shorter, so that the length of the duct as compared with that of the tubule proper is often as one to one, or even as two to one. They divide more frequently, so that often one duct will end in quite a cluster of short tubules. The tubules have a comparatively wide lumen, and are lined with chief cells only, the parietal being entirely absent (*Fig. 114*).

Surrounding the gland tubules, and extending between them and the muscularis mucosæ, is a delicate *connective tissue* of the adenoid type, with lymph corpuscles in its meshes. The surface of this tissue, *i.e.*, that part of it in contact with the glandular epithelium, is formed into a basement membrane by condensation of its reticulum and flattening of its superficial connective tissue cells. This tissue supports a capillary network derived from the larger vessels of the submucosa, and strands of non-striped muscle, which pass vertically between the glands, from the muscularis mucosæ.

Outside the vertically placed glands with their connective tissue basis, the *muscularis mucosæ* forms a narrow, complete layer, separating the mucosa from the submucosa. It consists of two layers of non-striped muscle, an inner arranged circularly, and an outer longitudinally. In some animals, as in the case of the cat, there are, however, three layers. In this animal, too, there is a clear homogeneous band (*Fig. 113 f*) immediately inside the muscularis mucosæ, which is not found in man.

As in the œsophagus, so here, the mucous membrane is thrown into rugæ or folds in the contracted state of the organ, and only forms a flat lining when the latter is distended, the loose nature of the submucosa affording facility for free movement between the mucous and muscular coats.

FIG. 113.

V.S. CARDIAC END OF STOMACH OF CAT, STAINED WITH PICRO-CARMINE \times 300.

- a.*—Duct of gland.
- b.*—Gland tubule.
- c.*—Gland tubule cut transversely.
- d.*—Muscularis mucosæ sending,
- e.*—Muscular strands between the gland tubules.
- f.*—Clear homogeneous layer.
- g.*—Central or chief cells.
- h.*—Parietal cell.

FIG. 114.

V.S. PYLORIC END OF STOMACH OF CAT, STAINED WITH HÆMATOXYLIN AND EOSIN \times 300.

- A.—Mucosa.
- B.—Submucosa.
- C.—Muscular coat.
 - a.* —Ducts of glands.
 - b.* —Gland tubules.
 - b.*¹—Gland tubule cut across.
 - c.* —Muscularis mucosæ.

Fig. 113.

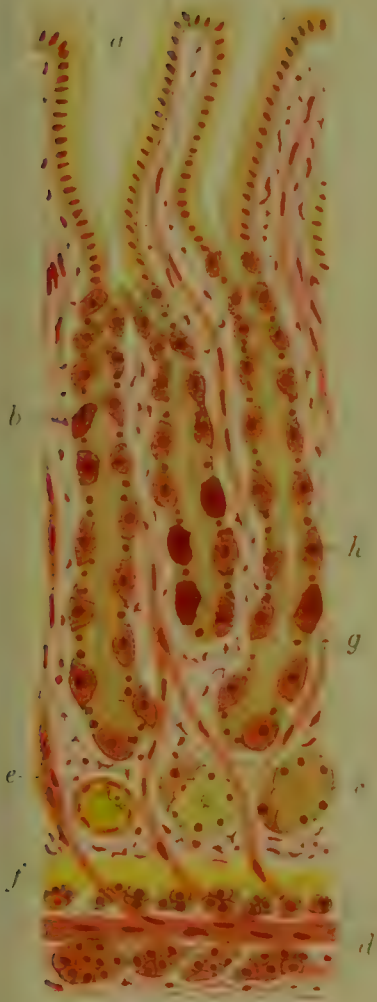
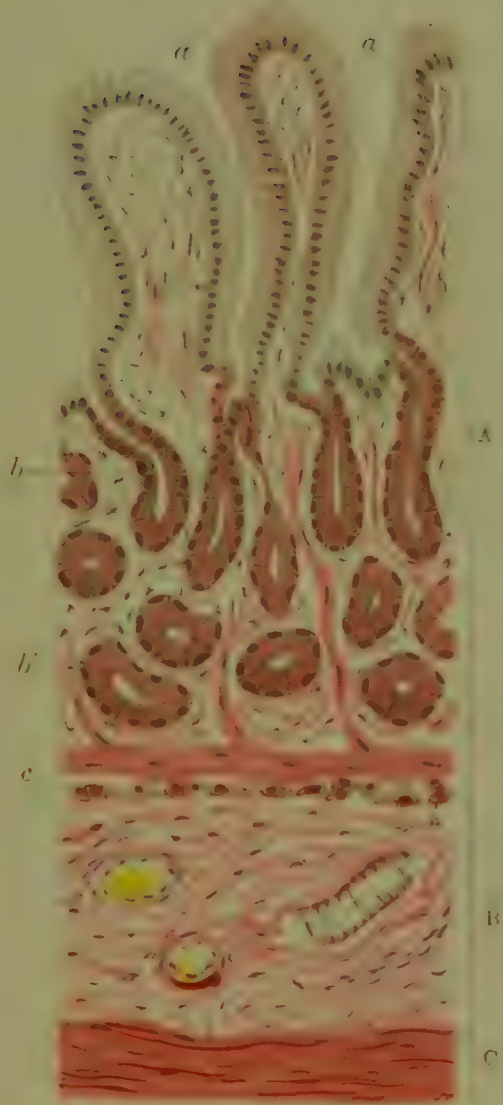


Fig. 114.



The submucosa consists of loose areolar tissue, and contains the larger blood-vessels and lymphatics. The large arteries give off branches which pierce the muscularis mucosæ, and run vertically between the glands, which they surround with capillaries. These form a network beneath the basement membrane, and are collected into venous trunks, which follow the same course as the arterial ones, and, piercing the muscularis mucosæ, reach the large veins of the submucosa. Outside the submucosa is the **muscular wall** of the stomach, consisting of two layers, an internal circular and an external longitudinal. The inner part of the circular layer is, however, more or less oblique, and may be separately mentioned. At either orifice of the stomach the circular fibres are increased in numbers, so as to form a sphincter, which is more distinctly marked at the pylorus. Outside the muscular coat is the **peritoneal covering**. This consists of a thin layer of connective tissue, forming a sheath for the external muscular coat, covered by a layer of simple squamous epithelium—polygonal nucleated squames, cemented together by their edges.

Examine the following specimens :—

(1,) *V. S. Cardiac end of stomach of cat, stained with methyl-blue.*
B.

(2,) *V. S. Cardiac end of stomach of cat, stained with picro-carmin.* F. (Fig. 113.)

Use the methyl-blue preparation for inspection under the low power, and that stained with picro-carmin for the high power.

Under the low power identify the different layers. Observe the broad mucosa, composed principally of the glands with their short wide mouths and longer tubular bodies. These tubes are marked out very distinctly in this specimen by the parietal cells, which take on the methyl-blue deeply. Outside the glandular part of the mucosa, note the muscularis mucosæ sharply dividing it from the rest of the wall of the stomach. With the high power examine first the epithelium of the mouth of the follicles (α), noting the length of the columnar cells and the clear nature of the outer half of them. In some places where the section has passed favourably, a surface view of the epithelium, lining the mouth, will be seen. Observe the hexagonal mosaic formed by the outlines of the cells. Note the point of commencement of the deeply stained parietal cells in the neck of the gland. The student will usually find that at first the only

cells which are very obvious to him in the body of the glands are these parietal cells, the central or chief cells being very much less distinctly seen. Observe that the parietal cells do not form a continuous layer, but are placed at intervals amongst or outside the less deeply stained central ones. Observe the shape, size, and relative number of the parietal cells at different parts of the tubule; that they reach the lumen in the neck, where they are smaller and more numerous; but that in the lower part of the body where they are fewer and larger (*h*), they are invariably outside the chief cells (*g*), between them and the basement membrane.

Notice that the bodies of the glands are not usually perfectly straight, but whether divided or single, they curl more or less towards their blind extremity. Between the lower extremities of these longitudinally cut glands and the muscularis mucosæ, look for transverse sections (*c*) of longer tubes, which have been thus cut in consequence of the bend upon them just mentioned. These transverse sections are very instructive. They show the small size of the lumen, the presence of the chief or central cells, and the relative position of the parietal cells very well. They have some resemblance at first sight to sections of mucous alveoli, the parietal cells representing the demilunes. But the central cells of a mucous alveolus are more transparent, and the demilunes are not the same shape as the parietal cells. These latter are more oval, and less half-moon shaped, and tend to cause a greater bulging outwards at the points where they occur. One, two, or three may be seen in a transverse section of a tubule. Look for the basement membrane outlining the tubules. The nuclei of the flattened cells can usually be seen here and there deeply stained by the reagent. Outside the basement membrane observe the delicate connective tissue, in which the glands are embedded. The blood capillary network is not seen in this specimen, but here and there strands of non-striped muscle fibres (*e*), derived from the muscularis mucosæ (*d*), may be seen running vertically between the gland tubules. Trace these strands into the muscularis mucosæ itself. Note that the latter, in the cat, is arranged in the form of three layers, and that there is a homogeneous clear band (*f*) just internal to them between the muscularis mucosæ and the rest of the mucosa, which is not found in man. Study the transverse sections of non-striped muscle in the muscularis mucosæ.

The loose connective tissue of the submucous coat presents no special feature. The larger blood-vessels and lymphatics may be noted in this layer,

The muscular coat often affords an excellent illustration of T.S. non-striped muscle. Of course, it will depend upon the direction in which the section has been cut as to which division is cut transversely. Note the irregular mosaic presented by the small transverse sections of the fibres, in some of which the centrally placed nucleus can be seen.

(3.) *V. S. Injected stomach of cat unstained. B.*

With the low power note the large vessels of the submucosa, giving off branches which pierce the muscularis mucosæ, and run vertically between the tubules. Here they give rise to a close capillary network, which is very definite round the lower end of the mouths.

The longitudinally cut division of the muscular coat affords a good illustration of injection of non-striped muscle. Contrast the smallness of the blood supply with that of voluntary muscle.

(4.) *V. S. Pyloric end of stomach of cat, stained with hæmatoxylin and eosin. B. (Fig. 114)*

With the low power note the mucosa (A), not so thick as at the cardiac end, and clearer—that is, it transmits the light more readily, due to the absence of the parietal cells from the tubules. Note the large size of the mouths of the follicles (a), and the comparative shortness of the tubular portion (b). Immediately within the muscularis mucosæ (c), observe the numerous transversely divided tubules (b'). With the high power note the epithelium of the mouths of the glands similar to that at the cardiac end. The bodies of the glands afford a comparatively easy study of the chief cells, which are here found alone, the parietal cells being completely absent. In the transverse sections of the tubules observe the comparatively large lumen.

In other respects, the pyloric end of the stomach is very similar to the cardiac.

THE SMALL INTESTINE.

The small intestine consists of the following layers from within outwards :—

(1.) *The Mucosa.* — (a,) *Epithelium* (Lieberkühn's glands, villi); (b,) *Connective tissue*; (c,) *Muscularis mucosæ*.

FIG. 115.

V.S. SMALL INTESTINE OF CAT, THROUGH PEYER'S PATCH, STAINED
WITH HÆMATOXYLIN AND EOSIN $\times 60$.

A.—Mucosa.

B.—Submucosa.

C.—Muscular coat.

a.—Villus.

*a.*ⁱ—Central lacteal.

*a.*ⁱⁱ—Watney's node.

*a.*ⁱⁱⁱ—Strands of muscle fibres from muscularis mucosæ.

b.—Lieberkühn's follicle.

c.—Muscularis mucosæ, giving off strands to villi.

d.—Epithelium covering,

e.—Lymph follicle of Peyer's patch.

f.—Connective tissue of submucosa.

Fig. 115.



(2,) *The Submucosa.*

(3,) *The Muscular Coat.*

(4,) *The Peritoneal Investment.*

The *epithelium* of the *mucosa* of the small intestine, like that of the stomach, is arranged in the form of a number of tubular glands placed vertically side by side. But, in addition to this, the intervening ridges are very frequently prolonged upwards, as finger-like projections or *villi*, whose main function is an absorptive one. As the epithelium of the alimentary canal is continuous throughout, that covering the villi passes directly into that lining the gland tubules (or *vice versâ*), and both are continuous with that of the pyloric end of the stomach (*Fig. 97*).

The naked eye appearance of the mucosa is distinctive. Like that of the stomach, it is thrown into a number of folds, in this case running transversely, the *valvule conniventes*, which project into the lumen of the gut. The central part of each of these folds is occupied by a prolongation of the loose connective tissue of the submucous coat. The folds commence at a little distance from the pylorus, and begin to disappear about the middle of the ileum. But in addition to these folds of the whole mucosa, the entire inner surface is beset with minute villi, which give it a velvety appearance. They are about 1 mm. in length, and .2 in thickness, and somewhat of the shape of a flattened finger. Solitary lymph follicles and collections of them are also to be found scattered throughout the small intestine. Individually they form small whitish projections, about the size of a pin's head. They occur as agminated patches of ten or twelve (Peyer's patches), especially opposite the line of mesenteric attachment in the ileum.

The glands which are known as *Lieberkühn's follicles* may be likened to a series of test-tubes placed side by side. They are lined by a layer of short, columnar granular cells, with a rounded nucleus placed near the base. Goblet mucin forming cells are to be found here and there amongst the columnar granular ones. The lumen of the tubule is more distinct than that of the gastric follicles. The glands are simple, and are not divisible into a mouth, or duct, and a body. The ridges between the tubules are covered with similar cells to those lining them. When the ridge is prolonged into a villus, the epithelium is still continuous, but changes somewhat in character. Lieberkühn's follicles secrete the *succus entericus*.

A *villus* consists of a core of connective tissue, containing blood-vessels, etc., covered with a layer of epithelium, continuous with that of the glands of Lieberkühn surrounding it. The epithelium consists for the most part of a single layer of tall columnar, granular cells, placed palisade-wise side by side. They have a vertically striated, refractile border at their free ends, and an oval nucleus placed in the basal third, its long axis in that of the cell. The striated hem of the cells placed in series gives the appearance of a bright border to the epithelial layer as a whole. This refractile band gradually fades off upon the surface of the cells lining Lieberkühn's follicles. Here and there, amongst the columnar cells covering the villus, but in much fewer numbers, are to be seen goblet, mucin forming cells. The large cup of the goblet contains transparent mucin, and the stalk a small amount of protoplasm, containing a compressed nucleus (*Figs. 115 and 116*).

Between the lower, narrower ends of the columnar cells, where they rest on the basement membrane, smaller cells frequently occur. They have a round nucleus and little perinuclear protoplasm, and are probably lymph corpuscles which have migrated from the interior of the villus.

The epithelium, both of the glands of Lieberkühn and of the villi, rests upon a basement membrane formed of a surface condensation of the *connective tissue* basis of the mucosa. It consists partly of expanded fibres, and partly of flattened connective tissue cells. It gives a distinct basal outline to the epithelial layer, which is especially well seen when the cells have been accidentally torn away. Here and there the deeply stained, flattened nuclei of the cells, taking part in its formation, help to demonstrate the basement membrane.

The connective tissue basis of the mucosa is somewhat of the adenoid type. The network is somewhat sponge-like, that is to say, the strands forming it are often plates rather than fibrils. Nuclei of connective tissue cells are to be seen at the nodes, and the meshes contain lymph corpuscles. This tissue forms a supporting framework for the glands of Lieberkühn, and for the important structures of the core of the villi. In addition to the glands of Lieberkühn it supports the capillary network around them, and the strands of muscular fibres passing upwards to the villi from the *muscularis mucosæ*.

The core of a villus is constituted as follows: In the centre is

the *lacteal*, a somewhat finger-like space, ending blindly, and enclosed by a layer of epithelial plates with a sinuous outline. It is, in fact, a lymph capillary, and is continuous with the lymphatics, more deeply placed in the mucosa. The space between the central lacteal and the basement membrane beneath the epithelium, is occupied by the retiform tissue above described, by blood-vessels, and by muscular fibres. The blood-vessels consist of a small artery, which splits up to form a capillary network, ending in one or two small veins, which return the blood to the larger vessels of the submucosa. The muscular fibres are derived from the *muscularis mucosæ*, and usually spread outwards as they approach the end of the villus, to become attached to the basement membrane. They may, however, terminate in the network of connective tissue. Their function is to shorten the villus periodically during the process of absorption.

Below (or outside) the blind ends of Lieberkühn's follicles, and separated from them by a narrow layer of retiform tissue, the *muscularis mucosæ* pursues an even course. It consists of two layers of non-striped fibres, an internal circular, and an external longitudinal. Strands pass vertically upwards from it between the glands of Lieberkühn, to terminate in the villi, and it gives passage to blood-vessels and lymphatics.

In the duodenum, beyond the pylorus, Brunner's glands are found. Their long ducts pass downwards from between the villi, pierce the *muscularis mucosæ*, and divide into the terminal acini in the submucous coat. These are lined by short columnar granular cells, similar to those of the glands of the pyloric end of the stomach. The ducts are lined by somewhat similar cells.

The **submucosa**, the **muscular coat**, and the **peritoneal investment**, require in themselves no special description. The muscular coat has but two divisions, an internal thicker circular one, and an external thinner longitudinal. Otherwise they resemble the same layers in the stomach.

Between the circular and longitudinal divisions of the muscular coat is found a remarkable plexus of nerve fibres, *Auerbach's plexus*. The strands forming the meshes are composed of non-medullated fibres, and at the junctions of the strands are collections of small nerve cells.

A somewhat similar network, though a finer one, called Meissner's plexus, exists in the submucous coat.

Throughout both the small and large intestines, *solitary*

FIG. 116.

V.S. VILLUS OF CAT'S INTESTINE, INJECTED, STAINED WITH HÆMATOXYLIN $\times 300$

- a.*—Layer of columnar epithelial cells.
- b.*—Interior of villus.
- c.*—Striated hem of epithelial cells.
- d.*—Adenoid reticulum.
- e.*—Mucous cell.
- f.*—Watney's node.
- g.*—Network of capillaries filled with red injection.
- h.*—Central lacteal of villus.
- i.*—Strand of non-striped muscle fibres.
- k.*—Nuclei of basement membrane.

FIG. 117.

V.S. SMALL INTESTINE OF RABBIT, INJECTED WITH BLUE GELATINE MASS $\times 50$.

- A.—Mucosa.
- B.—Submucosa.
- C.—Muscular coat.
 - a.*—Injected villi.
 - b.*—Artery or vein of villus.
 - c.*—Capillary network of villus.
 - d.*—Vessels of muscular coat.
 - e.*—Large vessel in submucosa.

Fig. 116.

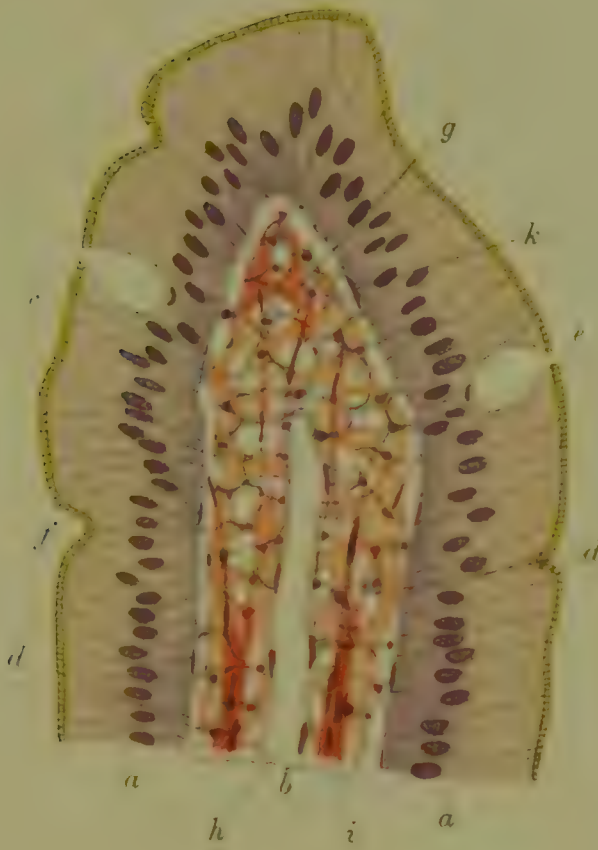
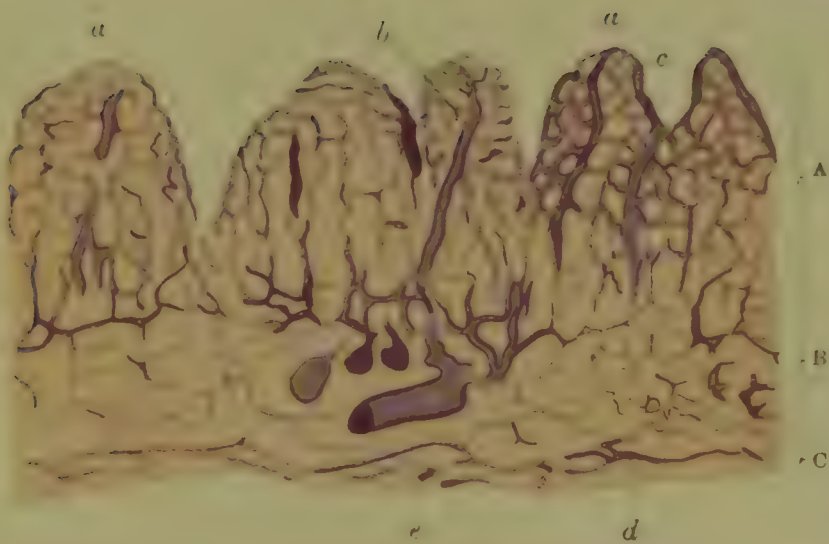


Fig. 117.



follicles occur. In the small intestine these are found either singly, or collected in groups, as in *Peyer's patches*. They usually have their base or broader part in the submucous coat, their apex projecting upwards through the muscularis mucosæ, and reaching the level of the junction of the villi with the glands of Lieberkühn. They are thus covered by a single layer of epithelium, with which, but for the basement membrane on which it rests, they are in direct contact. A solitary follicle consists of a fine reticulum of adenoid tissue, containing numerous lymph corpuscles, the whole surrounded by a lymph space in connection with neighbouring lymphatic channels. It possesses an afferent and efferent blood-vessel, and between the two a fine capillary network pervades the mass. (See page 233.) In the large intestine the follicles are smaller, and occur especially in the submucosa.

Examine the following sections :—

(1.) *V. S. Small intestine of cat, stained with hæmatoxylin and eosin. F. or B. (Fig. 115.)*

In examining sections of the stomach, or intestine, always place them so that under the microscope the lumen of the viscus—and consequently the mucosa—is away from you. In this case the fringe of villi renders it easy to detect the mucosa side of the section with the naked eye. Place it on the stage of the microscope with the fringe towards you, so that on looking through the tube the villi will point in the reverse direction.

Under the low power observe first the shape of the villi as shown in the figure. They are more or less club shaped, that is to say, they widen out towards their extremity, but narrow again to a point as the end is reached. The epithelium covering them is readily made out as a distinct layer from the core, by the nuclei of the cells placed side by side a little above the basement membrane. Observe that the contour of the surface of the villus is not quite regular, but is depressed here and there. These depressions indicate the presence of *Watney's nodes*, the nature of which will be better seen under the high power. Even under this power the refractile border of the epithelium as a whole, due to the striated hem of the cells, can be distinctly seen.

The structure of the core of a villus cannot properly be made out under the low power. The central lacteal may, however, be seen as a longitudinal space (a'), and on either side of it longi-

tudinally running strands should be noted, the prolongations from the muscularis mucosæ (α'''). These are usually to be seen in any villus, as they cannot fail to be cut, but the section may pass to one side of the central lacteal, which may thus not be visible. The rest of the core appears under this power to be composed of a number of nuclei, stained deeply with the reagent. Some are the nuclei of the connective tissue cells, others of the lymph corpuscles, others of the cells forming the walls of the capillaries.

Extending between the bases of the villi and the muscularis mucosæ, note the broad, deeply stained layer of the glands of Lieberkühn (b), placed parallel to each other and vertical to the surface. The muscularis mucosæ (c) is as usual a thin layer, forming a distinct dividing line between the mucosa (A) and submucosa (B) beneath. Note that it runs parallel with the lower ends of the follicles of Lieberkühn, and that when the mucosa is thrown into folds (this may or may not be seen in any individual section), the muscularis is folded with the rest of the inner coat, the submucous connective tissue running up into the fold in the form of a connective tissue core. Note the less deeply stained submucosa, a much narrower layer than the mucosa, and outside it the muscular coat (C) with its broad internal division and narrow outer one.

If the specimen shows a solitary gland (e), note its position. It occurs as a granular looking, deeply stained patch, in the submucosa alone, or extending to the surface of the mucosa. This latter is especially the case where the follicle forms one of an agminated group, or Peyer's patch. Note that such a follicle may only be separated from the cavity of the gut by the layer of epithelium (d).

Under the high power, study especially the structure of the villi and Lieberkühn's follicles. Examine first a villus (*Fig. 116*). Select one that shows the central lacteal in section. It must be noted that the amount of detail, which can be made out, will depend entirely upon the nature of the section. Thus, a specimen which shows the general arrangement well under the low power may be too thick to show the more minute structure of the different parts under the high. For examination with the high power a very thin section is required, and it is not of much consequence if the villi are displaced or broken as long as some of them are sufficiently intact to show their structure.

When the sections are thin the muscular coat is apt to become separated from the rest of the specimen.

Examine first the epithelium (*a*), noting the refractile border (*c*) of the tall columnar cells, the shape and position of the nuclei, and the presence here and there of goblet cells (*e*); lymph corpuscles also from the core of the villus are sometimes to be seen between the epithelial cells. The cup of a goblet cell appears somewhat as an egg-shaped space amongst the columnar cells. When the epithelium is seen in surface view, the goblet cells appear as round, unstained spaces, surrounded by the hexagonal mosaic of the columnar cells. The diameter of the round space varies, of course, with the level of the focus.

Find a depression in the epithelium indicating one of Watney's nodes (*f*). They indicate points in the layer where reproduction of the cell elements is taking place. Observe that in consequence of the depression the epithelium at this point is not so broad as it is elsewhere, and the cells in the immediate neighbourhood arch over it (*f*). Observe the basement membrane, a mere line, on which the epithelial layer rests. Here and there in the course of it are to be seen the flattened nuclei (*k*) of the connective tissue cells, of which it is partly composed. Now study the interior of the villus. Find first the central lacteal space (*h*), bounded by epithelial plates in section, with their flattened nuclei stained with the hæmatoxylin. On either side of the space look for an appearance of longitudinally running strands, which usually spread outwards towards the end of the villus, and can often be traced to the basement membrane, to which they are attached. These strands of non-striped muscle (*i*) are stained red with the eosin, but their rod-shaped nuclei are stained with the hæmatoxylin. Draw out the tube of the microscope to its full extent, and determine the character of the connective tissue basis. Look for an appearance of a network of delicate branching cells (*d*), many of the branches being flattened rather than fibrillar, with a blue stained nucleus in the body of the cells, *i.e.*, at the nodes of the network. In the meshes of the reticulum small round cells with distinct nuclei, but little perinuclear protoplasm, are to be seen. These are lymph corpuscles. The capillary network (*g*) is not well seen in an uninjected specimen. Many of the blue stained nuclei in the core of the villus are, however, those of the vessel walls.

Examine the crypts of Lieberkühn. Note how the refractile

FIG. 118.

V.S. LARGE INTESTINE OF CAT, STAINED WITH PICRO-CARMINE $\times 100$.

A.—Mucosa.

B.—Submucosa.

C.—Muscular coat.

D.—Peritoneal coat.

a.—Lieberkühn's follicle.

b.—Inner layer of muscularis mucosæ.

c.—Outer " " "

d.—Inner circular division of muscular coat.

e.—Outer longitudinal " " "

FIG. 119.

S. RABBIT'S LIVER, PORTAL VEIN INJECTED WITH CARMINE GELATINE MASS; BILE DUCTS INJECTED WITH WATERY SOLUTION OF PRUSSIAN BLUE $\times 45c$.

a.—Blood capillaries cut transversely.

b.—Nuclei of liver cells.

c.—Bile "capillaries" cut transversely.

(The cell network is shown in the right-hand part of the figure, omitted in the left.)

Fig. 118.

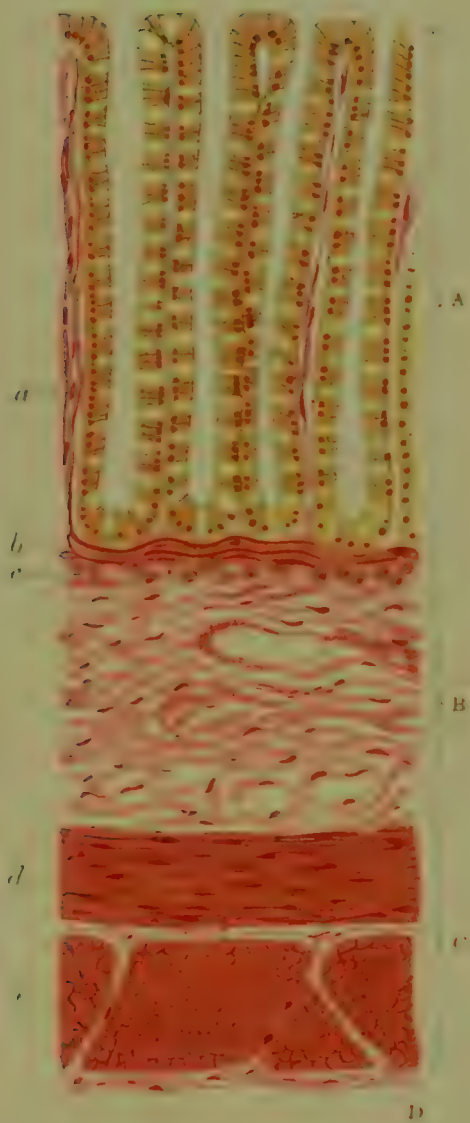
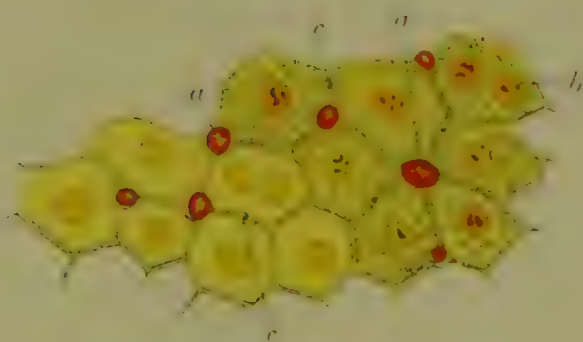


Fig 119.



border of the epithelium of the villus gradually becomes thinner, and fades off upon the surface of the cells of a follicle as it is traced down into it. Note, too, the transition from the very tall columnar cells covering a villus to the much shorter ones lining the gland tubes on either side of it. Observe that these are embedded in a basis of connective tissue, continuous with and similar to that of the villi, and that the cells rest on a similar basement membrane, with nuclei occurring in it here and there.

Examine the muscularis mucosæ, noting its two layers of non-striped fibres, the internal circular, and the external longitudinal. They will be cut in accordance with the direction of the section. If this has been in the long axis of the gut, the internal will be cut transversely, and the external in the line of its fibres. If, on the other hand, it has been made transversely to the long axis, the direction in which the fibres are cut will be reversed. Note the sections of single nuclei in the centres of some of the transversely divided fibres, and the rod-shaped nuclei in the layer which is cut longitudinally. Trace strands of fibres upwards as they pass between the follicles of Lieberkühn, to terminate in the villi. The rod-shaped nuclei, stained blue, of the cells of the fibres, render it easy to distinguish them. They are, moreover, stained red with the eosin.

Observe the structure of the submucous coat, which is similar to that found generally throughout the alimentary canal. That division of the muscular coat which is cut transversely affords here, as in the stomach, a very favourable opportunity of studying, some of the distinguishing characteristics of non-striped muscle. Note the smallness of the transversely divided fibres; the irregular mosaic they form in transverse section; the absence of connective tissue between them; the presence of a nucleus in the centre of some of them. This is, of course, due to the fact that many of the fibre cells are cut through either above or below the point at which the nucleus is situated. This accounts also for the difference in the size of the sections of the fibres, those with a nucleus being the largest.

Examine carefully the narrow layer of connective tissue between the two divisions of the muscular coat. It is easily recognisable from the muscular tissue on either side of it, as it is more lightly stained. When eosin has been used the muscle, being especially affected by this reagent, is still more easily distinguishable. In this thin septum of connective tissue look for

groups of nerve cells, round or oval, varying in size, each with a well marked nucleus and nucleolus. These groups are seen especially at points where the connective tissue is present in rather larger quantity, *i.e.*, where it runs up between two blocks of the transversely divided division of the muscular coat. Both Meissner's plexus occurring in the submucosa, and this which is Auerbach's plexus, will, however, be seen more satisfactorily in the gold chloride specimens about to be examined.

If one of the large solitary lymph follicles is present it will be seen to consist apparently of a mass of small round cells with large nuclei, and little perinuclear protoplasm. Observe the epithelium covering its surface, and note that the follicle is directly in contact with the epithelial layer (*Fig. 115, d*). Lymph corpuscles which have migrated from the mass of lymphoid tissue beneath are sometimes to be seen amongst the epithelial cells. This migration they are enabled to effect both here and in the case of the epithelium of the villi, owing to the imperfect character of the basement membrane which admits of their passage. The adenoid reticulum of the lymph follicle, and the lymph sinus round it, are not usually to be made out in an ordinary specimen.

(2,) *V. S. Pyloro-duodenal junction of cat, stained with hæmatoxylin and eosin. B. (See Fig. 97.)*

This section shows the transition from the pyloric end of the stomach to the duodenum. Under the low power observe that one half of the specimen shows the structure of the pyloric end of the stomach (*B 2*) as already described; the other the commencement of the duodenum (*Cj*). The transition from the one to the other is abrupt, and marked by the commencement of the villi, which appear immediately on the duodenal side of the *sphincter pylori* (*s*), formed by a well marked thickening of the internal circular division of the muscular coat. But, in addition to the villi, note the presence of Brunner's glands lying in the submucosa. This is thicker here than elsewhere in the intestine, in order to accommodate these glands. Under the low power they are seen as grape-like clusters of alveoli (*g*), the ducts passing from which pierce the muscularis mucosæ (*m*), and open on the surface between the villi. These groups of alveoli are to be seen commencing immediately on the duodenal side of the sphincter. At this point solitary lymph glands may also frequently be seen, sometimes in the mucosa, some-

times commencing in the submucosa, and piercing the muscularis mucosæ.

Note the enormous development of the internal circular division of the muscular coat, between the stomach and intestine, constituting the sphincter; the longitudinal portion is not thickened. Owing to *post mortem* contraction of the muscular coat, the section presents usually a convexity on the mucosal surface, and a concavity on the muscular. Under the high power study especially the glands of Brunner in the submucosa of the duodenum; also the transition from the pyloric glands to the epithelial lining of the intestine immediately on the duodenal side of the sphincter.

(3.) *V. S. Small intestine of rabbit, injected. B. (Fig. 117.)*

Examine the specimen for an appearance such as is shown in the figure. Each villus is supplied by an artery (*b*) springing from a larger vessel (*e*) in the submucosa. It passes along one side of the villus to reach the top, and a vein (or two veins) descend on the opposite side to join a larger vessel in the submucous coat. Note, in the villus, the very beautiful capillary plexus (*c*), between the artery and vein. It is not always feasible to recognise the artery and vein in the villus, but two vessels running longitudinally can usually be seen. Note the capillary plexus between the glands of Lieberkühn, and that the mucosa is the most vascular part of the intestinal wall, but that the larger vessels run in the submucosa. The longitudinally cut division of the muscular coat affords a good illustration of the vascularisation of non-striated muscle (*d*). Contrast its comparative scantiness with that of ordinary muscle.

(4.) *Muscular wall of rabbit's intestine (outer division), stained with gold chloride (for Auerbach's plexus). B. (Fig. 120 B.)*

This is a surface view of the plexus which usually adheres to the outer coat when the two muscular divisions are separated. The meshes of the plexus can be seen quite distinctly with the naked eye. Under the low power identify the somewhat quadrangular areas mapped out by strands of non-medullated nerve fibres, stained purple with the gold chloride. Note that the junctions of the strands show thickenings (*a*). In these thickened nodes minute light areas may be seen, round or oval, indicating the presence of the small nerve cells which are collected there. Notice the very delicate fibres (*b*) passing off from the main strands of the network to supply the muscular wall.

FIG. 120.

A.—MEISSNER'S PLEXUS, FROM MUSCULAR WALL OF RABBIT'S INTESTINE, STAINED WITH GOLD CHLORIDE $\times 80$.

B.—AUERBACH'S PLEXUS, FROM MUSCULAR WALL OF RABBIT'S INTESTINE, STAINED WITH GOLD CHLORIDE $\times 80$.

a.—Collections of nerve cells at nodes of network.

b.—Fibrillæ given off from main strands of network.

FIG. 121.

DIAGRAMMATIC REPRESENTATION OF COURSE OF BLOOD AND BILE IN LIVER.

a.—Bile ducts.

b.—Portal vein.

c.—Hepatic artery (part only shown, distribution omitted for the sake of clearness).

d.—Hepatic sublobular vein,

*d.*¹—Placed opposite central hepatic radicle of lobule, in which the course of the bile is shown.

*d.*²—Placed opposite central hepatic radicle of lobule, in which the course of the capillaries between portal and hepatic veins is shown.

e.—Tubules of liver lobule.

(The arrows indicate the direction of flow in the vessels against which they are placed.)

Fig. 120.

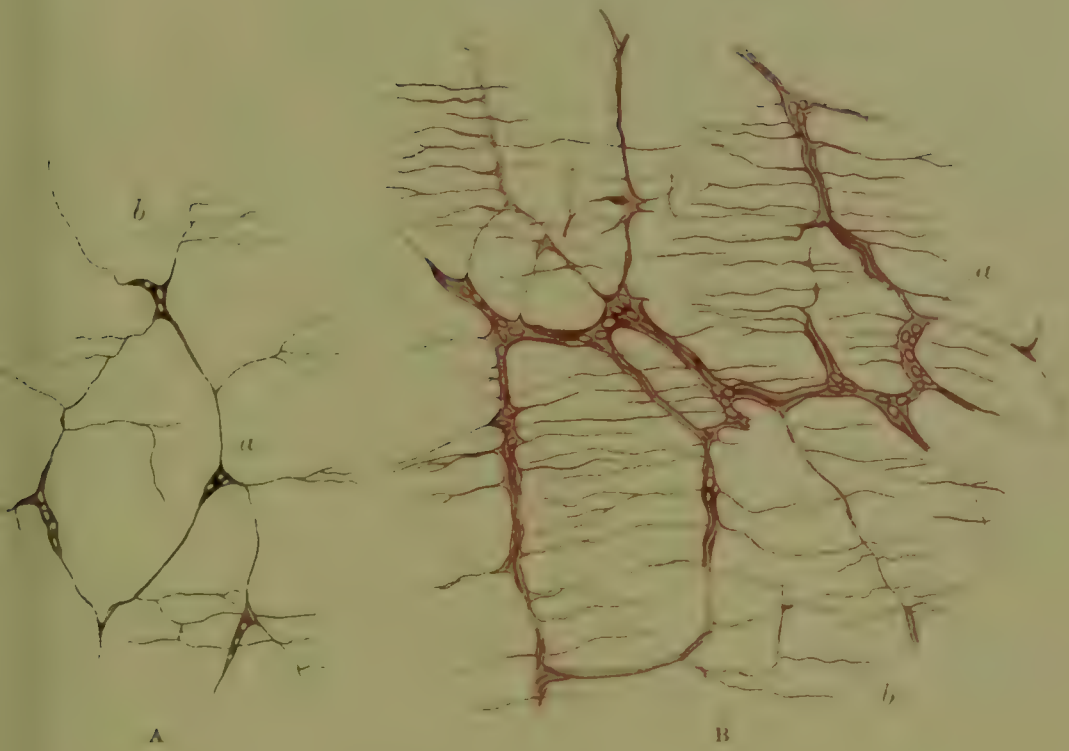
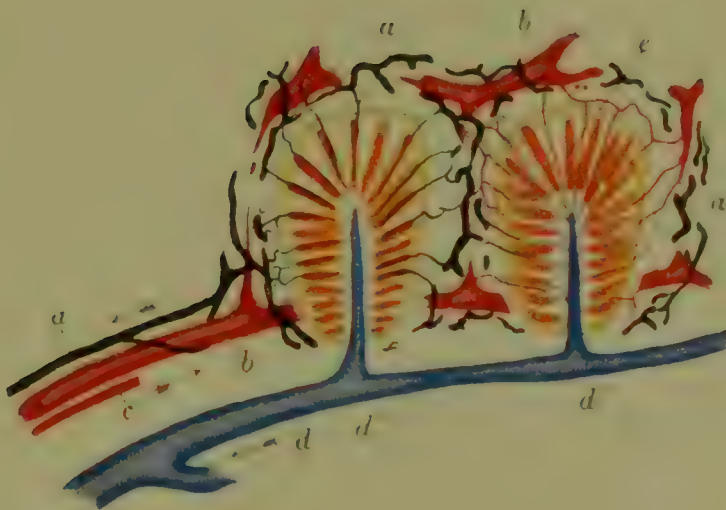


Fig. 121.



These fibres, as shown in the figure, run very largely parallel to each other, and at right angles to the main strands from which they are derived. Under the high power note the thickness of the strands, and that they are composed of several fibres ; the small nerve cells with unstained nuclei collected at the nodes ; the delicate fibres given off to the muscular wall.

(5.) *Muscular wall of rabbit's intestine (inner division) with part of submucous coat, stained with gold chloride (for Meissner's plexus). B. (Fig. 120 A.)*

This is a surface view of the submucous coat, or that part of it containing Meissner's plexus, supported by the internal muscular coat beneath.

Note the following points of difference between Meissner's plexus and Auerbach's.

In Meissner's plexus the meshes of the network are more irregular in shape ; they are not quadrangular as a rule, as is the case with Auerbach's plexus. They are smaller and the strands of the network are very much more delicate, often consisting of one fibre alone. The groups of nerve cells (*a*) are smaller. The secondary fibres (*b*) given off to the muscular coat, however, run parallel to each other in very much the same way as those in Auerbach's plexus.

(6.) *V. S. Intestine of frog, stained with osmic acid.*

This section is made from the intestine of a frog which has been fed with bacon the day previous to death, in order to demonstrate the passage of fat particles through the epithelium of the villi.

With the high power observe the globules of fat of all sizes, stained black with the osmic acid scattered through the epithelium covering the villi. Note that the fatty particles are in the cells themselves.

THE LARGE INTESTINE.

The large intestine differs from the small chiefly in the absence of villi and the restriction of the external longitudinal division of the muscular coat to three bands.

The epithelial element of the **mucosa** is represented entirely by the glands of Lieberkühn and the epithelium of the ridges between them. They are larger than in the small intestine, and their lumen is more distinct. The number of goblet cells

amongst the granular columnar ones is very largely increased. The cells covering the ridges between the glands tend to become elongated and narrower.

Lymphatic follicles (solitary glands) are frequent in the **submucous coat** of the large intestine. They do not usually pierce the muscularis mucosæ. Peyer's patches are absent.

The external division of the **muscular coat** is found as above stated in the form of three longitudinally running bands, between which the rest of the intestinal wall projects outwards in the form of **sacculi**.

Study the structure of the large intestine in the following section.

(1.) *V. S. Large intestine of cat, stained with picro-carmin.*
F. (Fig. 118.)

Under the low power note the absence of the villi, and that the mucosa (A) is composed almost entirely of Lieberkühn's follicles (*a*), placed vertically side by side. Note their distinct lumen.

Examine the muscular coat (C). This will vary in appearance according to the part from which the section was taken. Over the sacculi the inner circular division is thick; opposite the three longitudinal bands it is thin. The figure shows part of one of the longitudinal bands (*e*).

Under the high power study the cells of Lieberkühn's follicles, especially noting the very large number of goblet cells. Note that here there is no trace at all of a border to the cells, such as was prolonged in an attenuated form from the epithelium of the villi in the small intestine. Observe the lengthening of the cells upon the ridges between the glands.

THE RECTUM.

The **mucosa** of the rectum has very much the same structure as that of the large intestine. The external longitudinal layer of the **muscular coat** again becomes spread out uniformly over the viscus. It terminates abruptly at the anus. The circular division is here developed into the internal sphincter. At the anus the epithelium of the mucosa abruptly changes to stratified squamous.

THE LIVER.

The liver is a compound tubular gland. Its secretion enters the duodenum through the common bile duct at the same point as that of the pancreas. The cells of the tubules act not only as agents of secretion (bile), but also as store houses for a carbohydrate food material, which is brought to them by the portal vein from the alimentary tract, which is retained in them, and which is paid out again into the blood-stream in small quantities as it is required. It thus possesses a well defined *internal* as well as *external secretion*, in this way contrasting with others such as the salivary, and thyroid; in the former of which the secretion is mainly external, and in the latter internal. It is becoming increasingly apparent, however, that all glands influence metabolism otherwise than by their visible secretion. The vascularisation of the liver is peculiar to it, and the gland tubules are arranged in a special and characteristic manner.

The whole organ is invested with a fibrous envelope, *Glisson's capsule* (*Fig. 122 a*), which at the point of entrance of the bile duct and vessels affords a sheath to these structures, and supports them throughout their repeated subdivisions in the interior. When the surface of the liver, especially in some animals, is examined with the naked eye, it is seen to present a number of polygonal areas, about the size of a pin's head, representing the ultimate lobules of which it is composed. These areas have a darker central portion, and a lighter narrow periphery. The darker centre is composed of the tubules of the lobule, the lighter line, defining the polygons, indicates a system of connective tissue septa, bounding the lobules, continuous with Glisson's capsule on the one hand, and with the prolongation of it around the vessels and ducts on the other. These *interlobular septa*, in fact, correspond with the interlobular septa in the lung, which, it will be remembered, were connected in like manner with the pleural investment and the peribronchial sheaths. In other ways, too, there are marked resemblances between the arrangement of parts of the two organs, which will be shortly referred to; but the disposition of the tubules in a lobule itself in the liver is markedly different from that of the terminal air vesicles in a lobule of the lung.

A lobule of the liver is polygonal in shape, and is composed

FIG. 122.

S. LIVER OF PIG, SHOWING LOBULATION, STAINED WITH HÆMATOXY-
LIN $\times 20$.

- a.*—Glisson's capsule.
- b.*—Trabeculæ separating the lobules.
- c.*—A lobule.
- d.*—Portal tract, containing portal vein, hepatic artery, and bile ducts.
- e.*—Hepatic vein.

FIG. 123.

S. HUMAN LIVER, STAINED WITH CARMINE $\times 300$.

- a.*—Liver cells.
- b.*—Capillary wall, showing nuclei of epithelial plates.

Fig. 122.

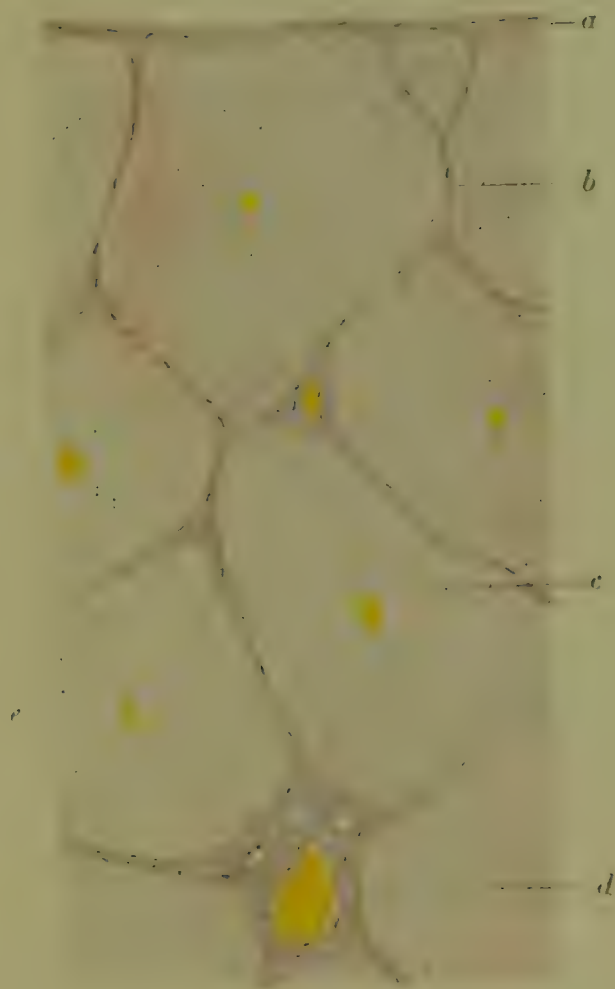
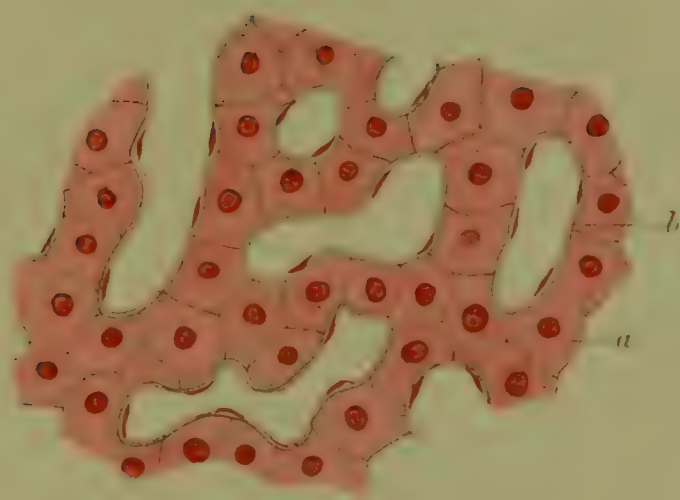


Fig. 123.



chiefly of a number of gland tubes, which radiate from near the centre of the lobule to the periphery, where they open into their ducts. Thus the blind terminal end of the tube is turned towards the centre of the lobule; the ducts at the periphery lie in the interlobular connective tissue, which to the naked eye marks the boundaries of the lobules.

The blood brought to the liver by the *portal vein* (Fig. 121, *b*) is conveyed along its subdividing branches till the ultimate subdivisions are reached, which lie together with the bile ducts (*a*) in the connective tissue surrounding the lobules. Here capillaries are given off which pierce the lobule, and pass between the radiating gland tubes (*e*) to reach the centre, where they open into the intralobular radicle (*d''*) of the efferent vein of the liver, the *hepatic vein*. These small hepatic radicles open into a larger vessel, the *sublobular vein* (*d*), and the sublobular veins unite to contribute to the hepatic vein itself. The walls of the branches of the hepatic vein are destitute of muscular fibres, and the adventitia is extremely thin.

The radiating gland tubes anastomose laterally with each other, as do the capillaries also. The meshes of the networks are elongated in a radial direction.

Thus a lobule is composed of a radiating system of gland tubes, and a corresponding radiating system of capillaries lying between them. A very minute quantity of connective tissue accompanies the capillaries as an adventitia, and in this lymphatic channels are to be found, separating the gland tubule from the blood-vessel.

The lobule is surrounded (in part or whole) with connective tissue supporting branches of the afferent portal vein, the feeder of the capillary network, and the *bile ducts*, which receive the secretion of the gland tubules. Thus the blood flows from the periphery to the centre of the lobule; the bile from the centre to the periphery.

But in addition to the afferent portal vein and the bile ducts, another vessel is found in the interlobular connective tissue. This is the *hepatic artery* (*c*), which supplies blood for the nutrition of the connective tissue of the organ, the vessel walls, etc. It ultimately terminates in the small portal veins, and perhaps partly in the capillaries in the periphery of the lobules.

Connective tissue framework of the liver.—It has been said that the portal vein, the hepatic artery, and the bile ducts (together

with lymphatics and nerves) lie in the intralobular connective tissue. They occur more particularly at the nodes of the fibrous septa network (*Fig. 122, d*), where the connective tissue is naturally in excess, and these are termed *portal tracts*, or spaces. Their shape depends, of course, on the contour of the lobules between which they lie, their size on the size of the vessels they surround. They contain very commonly a branch of the portal vein, recognisable from the largeness of its size and its patency; one or two sections of the ducts, very much smaller; sections of one or two branches of the hepatic artery, often smaller still; sections of nerves and lymphatics. A portal tract of the liver corresponds with a bronchial tract in the lung, and the following comparison between them may be useful:—

LUNG.						LIVER.					
<i>Bronchial Tract.</i>						<i>Portal Tract.</i>					
Bronchus	-	-	.	=	-	-	Bile duct.				
Pulmonary artery	-	-	.	=	-	-	Portal vein.				
Pulmonary vein	-	-	.	=	-	-	Hepatic vein.				
Bronchial artery	-	-	.	=	-	-	Hepatic artery.				
Lung alveoli	-	-	.	=	-	-	Tubules of liver.				

The connective tissue is of the same nature as that forming the supporting framework of other glands, *viz.*, white fibrous tissue with a small admixture of elastic fibres, with connective tissue corpuscles between the white fibres.

The connective tissue framework of the liver may be compared with that of the lung as follows:—

LUNG.						LIVER.					
Peribronchial connective tissue	-	-	-	-	=	Connective tissue of portal tract.					
Pleura	-	-	-	-	=	Glisson's capsule.					
Interlobular septa	-	-	-	-	=	Interlobular septa.					

The completeness with which the lobules of the liver are separated from each other by the septa of fibrous tissue, in other words, the extent to which the interlobular septa are developed, varies in different animals. It is very marked in the pig (*Fig. 122*), much less so in the human liver. A portal tract in the latter frequently bears some resemblance to a column fluted on three, four, or more sides, according to the number of series of lobules with which it is in contact; or to a series of tendon cells moulded conformably to the bundles of fibrous tissue, between which they lie, and between which their substance is prolonged

in the form of plate-like projections. In such a liver as the human, or that of the cat or rabbit, these plate-like projections are sometimes carried far enough to meet similar ones from neighbouring tracts, thus completely demarcating the lobules they enclose; sometimes they are only prolonged a little way towards those of their neighbours, with the result that the lobules appear to be not completely separated from each other in the intervening space.

Structure of the gland tubes.—In a form of liver simpler than the mammalian—such a one as the *frog's*—the tubules are not arranged in the form of lobules, but more diffusely throughout the organ. They constitute a branching anastomosing network, with which is intertwined a corresponding blood capillary one. The cells lining the tubes are large and wedge shaped, with a nucleus placed in the outer broader part, the apex of the wedge abutting on the narrow lumen. As the tubes are traced into their ducts the epithelium becomes first cubical and then columnar.

The liver of a mammalian, such as the *rabbit*, is, as we have seen, more complicated in its general arrangement. The gland tubules themselves anastomose more freely, and there is a greater difficulty in recognising their essential nature. In the frog, on the contrary, it is easy in an uninjected specimen to recognise the tubular character of the gland. In a section of mammalian liver we see only a series of radially disposed columns of polygonal nucleated cells; columns which anastomose freely with each other laterally, and which appear to terminate as abruptly at the periphery of the lobule in which they occur as they do towards its centre. In these columns the cells appear to be placed sometimes in single rows, and sometimes two abreast, according to the way in which the column is cut (*Fig. 123*).

At the periphery of the lobule the mass of gland tubes is sharply marked off from the connective tissue of the portal space, and in an uninjected specimen the connection between the gland tubes and the bile duct in the space is not seen. It consists, however, of a short intermediate ductule lined with low cubical cells.

The hepatic ducts leaving the liver, the cystic duct and gall-bladder, and the common duct, possess a mucosa consisting of a layer of columnar cells, with goblet cells here and there, resting upon a layer of connective tissue. In these situations mucous glands also are found in the mucosa. Outside is a muscular

FIG. 124.

T.S. PORTAL TRACT OF LIVER OF CHILD, STAINED WITH PICO-CARMINE $\times 250$.

- a.*—Portal vein.
- b.*—Hepatic artery.
- c.*—Bile duct.
- d.*—Liver cells.
- e.*—Capillaries between the tubules of the lobules.
- f.*—Fibrous tissue of portal tract.

4

FIG. 125.

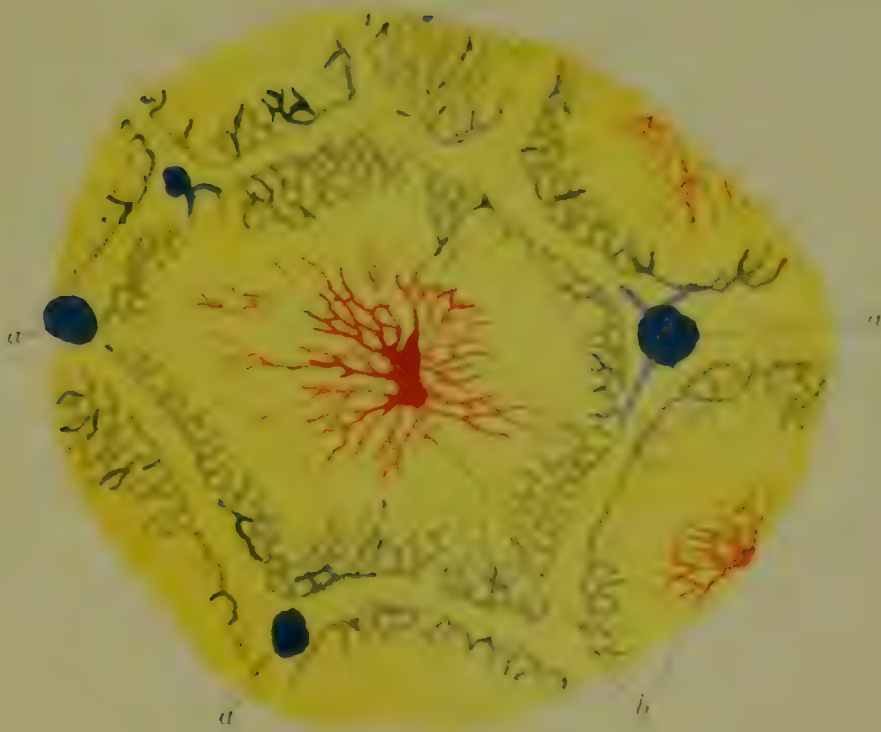
S. LIVER OF PIG, PORTAL VEIN INJECTED BLUE, HEPATIC VEIN INJECTED RED.

- a.*—Portal vein in interlobular connective tissue.
- b.*—Hepatic vein in centre of lobule.

Fig. 124.



Fig. 125.





coat, consisting of two divisions, an internal circular, and an external longitudinal. The larger interlobular ducts are lined by columnar epithelium resting on a connective tissue basis, containing smooth muscle fibres circularly arranged. As the ducts are followed in their subdivisions within the liver, the muscular coat diminishes in thickness, and finally disappears, the longitudinal division going first, so that in the small ducts in the smaller portal tracts we have merely a layer of columnar or cubical cells resting on a connective tissue basis. These smaller ducts give off anastomosing *intermediate ductules* lined by low cubical cells, which open into the tubules at the periphery of the lobules.

If the bile duct and portal vein are injected with different colours, the relations of the two capillary networks in the lobules are revealed, and also the connection between the ducts of the portal spaces and the gland tubules through the intermediate ductules. *Fig. 126* shows a portal vein (*a*) and a bile duct (*b*) in the portal tract; and springing from these, two networks are to be seen; a blood capillary network (*c*), comparatively large and coarse, and a bile "capillary" network (*d*), which is much finer. The bile "capillary" network represents, of course, the lumina of the anastomosing gland tubules. The two networks are invariably separated from each other by at least a portion of a gland cell. When the section has cut the capillaries transversely (*Fig. 119*), the large round sections of the blood-vessels (*a*) may be seen situated at the angles of the cells, whereas the bile capillaries (*c*), showing merely as points, are seen in the middle of the adjacent sides of the gland cells; that is to say, that each of the two adjacent cells is grooved by the bile lumen along the middle of the side in contact with its neighbour. As before stated, in this more highly differentiated mammalian liver the typical relation between the blood-stream and the secretion of a gland is somewhat masked by the complexity of the arrangement of the gland tubules. But the principle of construction is precisely the same as in other glands, the lumen of the tubules being separated from the blood and lymph without by the intervention of the secreting epithelium.

Here, however, the lumen of the tube is not surrounded by four, five, or more cells, but lies between two, the opposing surfaces of which are consequently grooved to admit of it; that is to say, the tubules of the liver are represented by columns of cells

placed two abreast, with the lumen of the tube lying between them.

The individual cells are polygonal in shape, and possess a round, centrally placed nucleus. The cell wall is slight, and represented by a condensation of the periphery of the protoplasm. The appearance of the perinuclear protoplasm, as in other secreting glands, varies from time to time. It contains granules, the precursors of secretion during the condition of rest, which disappear during the condition of activity. *Glycogen* is stored by the cells in the form of large hyaline granules, which stain a port-wine colour with iodine. They are found especially around the nucleus, spreading outwards throughout the cell if the animal is liberally supplied with carbohydrates, disappearing entirely if it is fed on proteid alone. If the glycogen granules are dissolved out with water a coarse network is left, the spaces in the protoplasm which they occupied appearing as vacuoles. The granules can be fixed either by alcohol or osmic acid.

Examine the following specimens :—

(1.) *S. Liver of pig, stained with hæmatoxylin. B. (Fig. 122.)*

Under the low power observe the polygonal, variously shaped lobules (*c*), marked out by the interlobular connective tissue (*b*). Look round the edge of the section, and at one side find a portion of Glisson's capsule (*a*) in continuity with the interlobular trabeculæ. Look for a portal tract (*d*), placed at the junction of several septa. Recognise in it one large opening, the portal vein, and one or more sections of the bile ducts and hepatic artery. The bile ducts are readily recognised by the deep staining of the nuclei of the columnar epithelial cells.

Look at the areas, the lobules mapped out by the trabeculæ. Observe that in this liver they are completely demarcated from each other; in other words, that the connective tissue network is continuous throughout the section. Even under this power the radial arrangement of the columns of cells in the lobules may be made out, and in the centre of many, a section of the hepatic radicle (*e*).

Put on the high power and examine the structure of Glisson's capsule, and the interlobular trabeculæ. Examine also the vessels and ducts in the portal space. It is quite possible, however, that in this specimen a portal tract is not well shown. The main purpose of the section is to give a general view of the

liver in an animal in which the lobular arrangement is very distinct.

(2,) *S. Liver of child showing portal tract, stained with hæmatoxylin or picro-carmin.* B. or F. (Fig. 124.)

Under the low power contrast the imperfect division into lobules as compared with the pig's liver. Find the best portal tract, cut transversely, in the section, and identify the structures contained in it—the large portal vein (*a*), the branches of the bile duct and hepatic artery (*c* and *b*), here and there sections of nerves, and lymphatic channels. Put on the high power and examine the structure of each of these separately. If the tract is a fairly large one look for non-striped muscular fibres running circularly in the walls of the vein and bile ducts. They are always found, of course, in the wall of the hepatic artery. Examine the columnar epithelium of the bile ducts (*c*). Note the nuclei of the epithelial cells lining the blood-vessels and lymph channels.

Now examine the columns of liver cells in a lobule (Fig. 123). Note that the cells (*a*) are frequently pentagonal or hexagonal, that they possess a centrally placed nucleus, and a distinct intracellular network. In some cells two nuclei may sometimes be seen. Look for the capillary network between the columns. It varies in size according to its distension with blood; if the capillaries are empty and collapsed they are encroached upon by the cells, and *vice versa*. Note the nuclei of the capillary walls (*b*).

Examine a transverse section of a hepatic radicle in the centre of a lobule. Note the extreme thinness of its wall, which consists merely of a layer of epithelial plates resting on a little delicate connective tissue, continuous with that surrounding the capillaries within the lobule.

(3,) *Isolated liver cells of rat, stained with picro-carmin.* F. (Fig. 14, A.)

Examine the isolated cells under the high power. Note their various shapes, hexagonal, pentagonal, irregularly polygonal. Each has a round nucleus, stained deeply with the carmine placed in the centre. The perinuclear protoplasm is stained with the picric acid, and to some extent with the carmine. Numerous blood corpuscles are also to be seen scattered over the field. They are easily distinguished from the liver by their different outline, and the absence of protoplasm around their oval nuclei.

FIG. 126.

S. LIVER OF RABBIT, PORTAL VEIN INJECTED RED, BILE DUCTS
INJECTED BLUE $\times 300$.

- a.* — Portal vein.
- b.* — Bile duct.
- c.* — Portal capillary network.
- c.*¹ — Capillary cut across.
- d.* — Biliary network.

FIG. 127.

SCHEME OF TUBULES OF KIDNEY.

- A. — Interpyramidal cortex.
 - B. — Pyramid of Ferrein.
 - C. — Cortex.
 - D. — Boundary layer
 - E. — Papillary region
- } of medulla.
- a.* — Malpighian capsule.
 - b.* — First convoluted tubule.
 - c.* — Spiral tubule.
 - d.* — Descending limb of Henle's tubule.
 - e.* — Henle's loop.
 - f.* — Ascending limb of Henle's tubule.
 - g.* — Irregular tubule.
 - h.* — Second convoluted tubule.
 - i.* — Junctional tubule.
 - j.* — Straight collecting tubule.
 - k.* — Discharging tubule.
 - l.* — Sinus of kidney.
- 1. — Radiating artery.
 - 2. — Afferent vessel to glomerulus.
 - 3. — Efferent vessel from „
 - 4. — Capillary network between tubules.
 - 5. — Glomerulus.

Fig. 126.

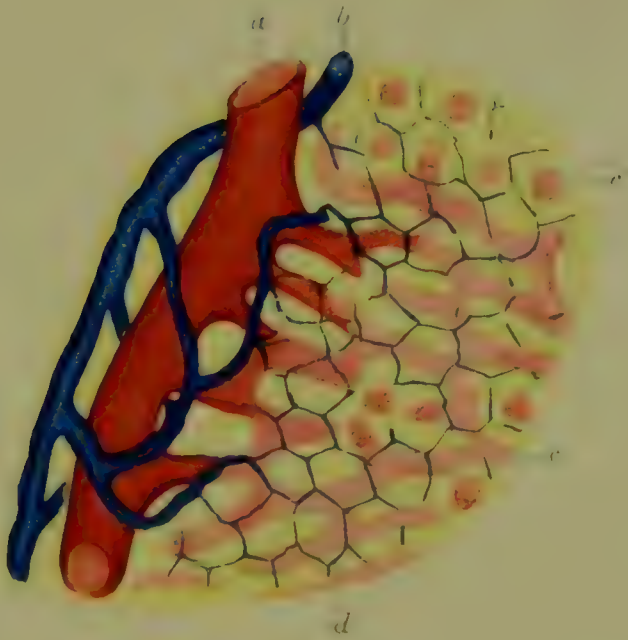
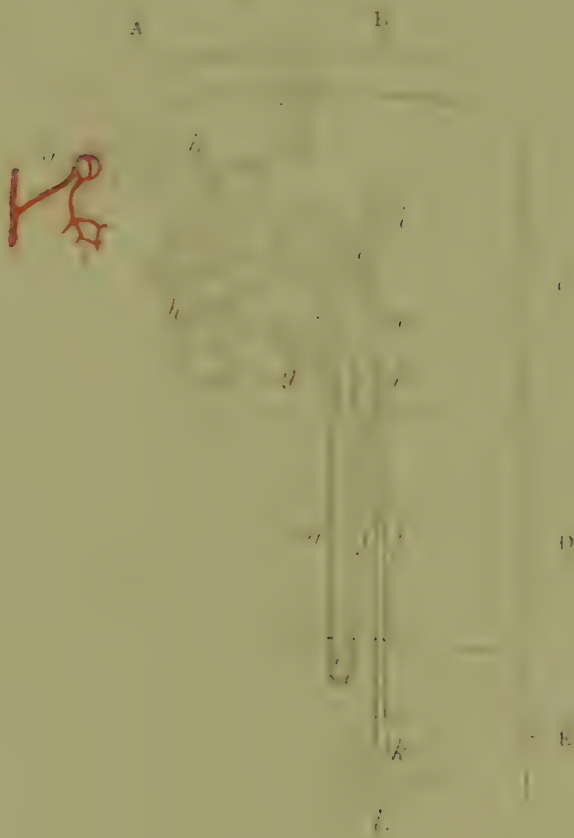


Fig. 127.



(4,) *Section of recently fed rabbit's liver, hardened in alcohol, stained with iodine. F.*

Under the high power examine the granules of glycogen, stained of a port-wine colour with the iodine, in the perinuclear protoplasm.

(5,) *S. Frog's liver, hardened in osmic acid, stained with hæmatoxylin. B.*

Under the high power study the tubular nature of the gland, which is easily recognisable. Note the narrow lumen of the anastomosing gland tubes with, in a longitudinal section, a single layer of large, somewhat quadrangular cells on each side of it. If a transverse section of a tubule is examined the lumen will be seen to be surrounded by three, four, or more cells, rather wedge shaped when seen from this point of view. Observe the nuclei placed in the outer part of the cells.

The intertubular capillary network is here very well seen. Note the line of the epithelial wall immediately outside the tubules, with a flattened, deeply stained nucleus occurring here and there. In the lumen of the capillary observe the elliptical, nucleated, biconvex, amphibian corpuscles.

(6,) *Liver of pig, portal vein injected blue, hepatic vein injected red. B. (Fig. 125.)*

This is frequently a very beautiful preparation. Under the low power note how distinctly the two areas, portal, occupying the connective tissue septa, and hepatic, occupying the centre of the lobules, are differentiated from each other. In a perfectly successful preparation the lobule is divided between the two; the outer part being injected blue from the portal vein, and the inner red from the hepatic vein. This ideal result cannot, however, always be attained.

The portal vessels are seen as round, oval, or cylindrical masses of blue injection (*a*), lying between the lobules; and from them a capillary network is given off to penetrate some distance into the interior, where it joins the network injected red from the central hepatic vein (*b*). If this central hepatic radicle should be cut longitudinally, its relation to its lobule, as shown in *Fig. 121*, may be seen, and perhaps its junction with the larger sublobular vein.

(7,) *Liver of rabbit injected through the portal system alone, with blue or red gelatine mass. B.*

In this case one injection is forced right through the

organ, so that the portal and hepatic systems are similarly affected.

(8,) *Liver of rabbit, portal vein injected with carmine gelatine mass; bile ducts with watery solution of Prussian blue.* (Fig. 126.)

Under the low power note the injection of the blood-vessels with red, as before. The portal tracts show also the bile ducts filled with blue injection (*b*), and thus reveal the anastomoses of the smaller branches and of the intermediate ducts. This anastomosis is well shown in the figure where the branches are seen surrounding the portal vein (*a*). Look for a fine hexagonal network of blue lines (*d*) extending from these anastomosing bile ducts into the lobule. It is easily distinguished from the coarser, red network of the blood capillaries with which it is intertwined (*c*). Put on the high power. Observe in many parts the two networks revealed in Fig. 126; but look also for an appearance here and there such as is shown in Fig. 119. Observe the transverse sections of the blood capillaries, large, circular, either filled with injection or empty, occurring at the angles between the cells (*a*); and small blue points, the transverse sections of bile "capillary" lumina, occurring between adjacent cell sides (*c*).

THE PANCREAS.

The pancreas is a compound racemose gland, the terminal alveoli of which are rather tubular than saccular in shape. Its secretion is poured into the duodenum through the duct common to it and the liver.

The gland is invested by a capsule of ordinary connective tissue, containing blood-vessels and lymphatics, which gives off septa into the interior, dividing it into lobes, and these again into lobules. The large blood-vessels and ducts run in the septa, and are distributed throughout the gland in the same way as in the salivary glands. The larger ducts, down to the small intra-lobular branches, are lined by columnar epithelium, which is striated, though not so markedly as in the salivary glands. The small intermediate ducts terminating in the alveoli themselves, are lined by flattened cells. These intermediate ducts in the pancreas (Fig. 111, *a*) are longer and more delicate than those of a mucous gland.

The alveoli themselves are tubular in form. The lumen is small, and is surrounded by cells in one layer, of the serous as opposed to the mucous type. The pancreas thus resembles the parotid rather than the sublingual gland. The cells are columnar or wedge-shaped, and rest on a basement membrane, which is continued around the ducts. They possess a spherical nucleus placed a little to the outside of their middle. The cells show two zones, more distinct than those seen in the parotid, an outer one finely granular (*f*), purely protoplasmic which stains deeply with the reagent employed; and an inner one (*e*) less deeply stained, which is coarsely granular. These inner granules represent the precursors of secretion as in the parotid cell. The relative preponderance of these two zones naturally depends on the stage of secretion. If the cell is "loaded" previous to secretion, the non-staining refractive large granules may extend from the inner part outwards till they obscure the nucleus. When the gland is exhausted by secretion, this inner coarsely granular zone is very narrow, and is confined to that part of the cell immediately next to the lumen. On exhaustion, too, the cells become smaller, the outline of the alveolus becomes indented at the points where the bases of neighbouring cells touch, and the lumen becomes more distinct.

The secretory granules are not dissolved by hardening reagents in the same way that those of the parotid are.

As in the salivary glands, the alveoli are surrounded by a capillary network, supported by delicate connective tissue (*d*).

Here and there, in the interlobular septa, and between the alveoli themselves, are to be seen small groups of polygonal nucleated cells, invested with a capillary network. Their function is not known. "Centro-acinal" is the name sometimes applied to small cells found here and there occupying the lumen of the alveoli.

Examine sections of the pancreas of a dog, cat, rabbit, or guinea-pig:—

(I,) *Pancreas of dog, stained with borax-carmin.* B. (Fig. III.)

Under the low power note the general arrangement of the parts, and compare it with that of the salivary glands. Note that the alveoli are of the serous type. They are smaller than those of a mucous gland, more angular, do not transmit light readily, have no dark outline (absence of demilunes). Put on

the high power and examine the ultimate structure of the alveoli. Observe the outer, more deeply stained zone in the cells (*f*); the round nucleus and its position a little to the outside of the centre, rather than at the base as in a mucous cell; the absence of demilunes. Observe the large lightly stained granules of the inner part of the cells (*e*). Find an intermediate duct (*a*), and trace it into its alveoli (*b*).

Of course, if the gland has been resting, and is "loaded," the secretory granules of the inner zone will be found to have extended outwards, and may monopolise the cell. The completely exhausted condition is only seen in glands which have been stimulated through their nerve with the interrupted current for some hours.

(2.) *Pancreas of dog, injected with carmine gelatine mass.* B.

Observe the free vascular supply between the tubules and the capillary network investing the groups of polygonal cells.

APPENDIX TO CHAPTER XII.

METHODS OF PREPARATION.

1. The œsophagus.—Use the œsophagus of a dog or pig. Cut the tube transversely into pieces, half-an-inch long ; harden in Müller and spirit, cut in gum, stain in picro-carmin or hæmatoxylin and eosin, and mount in Farrant or balsam. Smaller œsophagi of cat, rabbit, or guinea-pig, may be stained in bulk in borax-carmin and cut in paraffin.

2. Stomach of cat.—Excise and open the stomach of a cat, and wash in methylated spirit. The cardiac end is readily distinguished from the pyloric by the presence of rugæ (foldings of the mucous membrane), and its greater thickness. Place pieces from each end in (1,) chromic acid and spirit (*page* 12) for a week to a fortnight ; (2,) Small pieces $\frac{1}{2}$ in. cube, in osmic acid, $\frac{1}{2}$ per cent., for 24 hours ; complete in spirit ; (3,) Müller and spirit ; (4,) spirit alone. Cut in gum, stain (*a*,) in methyl-blue and mount in balsam ; (*b*,) in picro-carmin and mount in Farrant ; (*c*,) in hæmatoxylin, or hæmatoxylin and eosin, and mount in Farrant or balsam ; (*d*,) pieces hardened in osmic acid may be mounted without further staining in Farrant or balsam. The tissue may be cut in paraffin instead of gum, in which case it may be stained in bulk before cutting, in borax-carmin or Kleinenberg's hæmatoxylin.

3. Injected stomach of cat.—Harden pieces of the cardiac end of the stomach of a cat, successfully injected with carmin or blue gelatine mass from the aorta, in Müller and spirit. Cut thick sections in gum, dehydrate and clear in watch-glasses ; mount in balsam. If desired, the sections may be stained in contrast to the injection before dehydrating. If the injection is red, they should be stained with hæmatoxylin ; if blue, with picro-carmin.

4. Pyloro-duodenal junction of cat.—Excise the pyloro-duodenal junction of the stomach of a cat ; slit the tube longitudinally ; wash in spirit, harden in chromic acid and spirit, or in Müller and spirit ; cut in gum, stain in hæmatoxylin, and mount in balsam.

5. Small intestine of cat.—Excise part of the small intestine of a cat ; slit it up, and wash in 50 per cent. spirit. Harden pieces in Müller and spirit, or chromic acid and spirit. Cut in gum, stain in picro-carmin or hæmatoxylin, or hæmatoxylin and eosin, and mount in Farrant or balsam ; or stain in bulk in borax-carmin, and cut in paraffin. In hardening it is advantageous to suspend the pieces separately in the fluid by means of threads, to avoid injury to the delicate villi. Change the fluid frequently.

6. Small intestine of rabbit or cat injected.—Excise, open, and wash in spirit, part of the small intestine of a cat or rabbit, which has been successfully injected from the aorta with carmine or blue gelatine mass. Cut into pieces; harden in Müller and spirit. Cut thick sections in gum or paraffin, and mount in balsam.

7. Auerbach's and Meissner's plexuses.—Excise a portion of the small intestine of a rabbit, and wash in normal saline, by allowing the fluid to pass through it. Close one end, and fill with gold chloride and formic acid solution (*page 47*: "boiled" method), and suspend in same for 10 to 15 minutes. At the end of that time, remove and wash in water and replace the gold chloride solution both inside and outside the tube with formic acid, 20 per cent. solution, in which it remains in darkness for 24 hours; preserve in spirit. In making a preparation of Auerbach's plexus, take a piece of the intestine, $\frac{3}{16}$ in. square, strip off the external muscular coat, dehydrate and clear it in watch-glasses, and mount in balsam, with the internal surface uppermost. Auerbach's plexus will be found adhering to it. To make a preparation of Meissner's plexus, take the remainder of the original piece, place it on a slide with the mucous surface uppermost, and with a scalpel gently scrape away the mucous and part of the submucous coat; inspect the remainder from time to time under the low power, and as soon as Meissner's plexus is well seen, dehydrate and clear in watch-glasses, and mount in balsam.

8. Frog's intestine for absorption of fat.—Feed a frog with a few pieces of bacon-fat overnight. The pieces are readily forced through the pharynx into the stomach by means of the handle of a needle. The next day, or, preferably, the second day, kill the animal; excise and open the small intestine; harden for 24 hours in 1 per cent. osmic acid, and imbed and cut in paraffin; or cut in gum, stain with picro-carmine and mount in Farrant. If the paraffin method be adopted, keep the tissue as short a time as possible in alcohol to dehydrate it, as the reagent dissolves the fat globules, and use chloroform in preference to turpentine as the intermediate stage between alcohol and paraffin. Do not transfer direct from chloroform to paraffin, but first to a mixture of the two.

9. Large intestine of cat.—Same as 5.

10. Liver of pig.—Harden in Müller's fluid, cut in gum, stain in picro-carmine or hæmatoxylin, and mount in Farrant or balsam.

11. Liver of child.—Same as 10, but spirit may be added to the Müller's fluid, as there is not the same danger of over-hardening, due to the smaller amount of connective tissue in the organ. For the same reason, pieces may advantageously be stained in bulk in borax-carmine and cut in paraffin.

12. Isolated liver cells of rat.—(See *page 122*).

13. Rabbit's liver for glycogen.—Feed a rabbit with carrots, and kill in a few hours' time. Very rapidly remove the liver; cut in small pieces and place in absolute alcohol; after 24 hours, wash in water. Cut in gum, and stain with weak iodine solution. Mount in Farrant.

14. Liver of frog.—Harden pieces of the liver of a frog in osmic acid for 24 hours. Complete in spirit. Cut in gum. Stain in picro-carmine or hæmatoxylin, and mount in Farrant or balsam; or stain in bulk in borax-carmine, and cut in paraffin.

15. Liver of pig injected.—The liver is injected in this case after removal from the body. First wash out the blood as far as possible by injecting water at the body temperature through the portal vein. Then inject the portal vein with blue gelatine mass, and the hepatic vein with red. It is not necessary, however, to inject the whole organ, but the large branch of either vessel supplying one particular area may be selected. Allow to cool. Cut the injected portion into $\frac{1}{2}$ -inch cubes, and harden in Müller's fluid. Cut in gum, and mount in balsam.

16. Liver of rabbit, injected with one colour.—Inject the animal through the aorta in the usual way, with either red or blue gelatine mass. Allow to cool. Cut the liver in pieces and harden in Müller and spirit. Cut in gum, and mount in balsam.

17. Liver of rabbit (portal vein injected red, bile ducts injected blue).—Inject the liver of a rabbit from the aorta with carmine gelatine mass. After injection, cool rapidly under the tap, and inject the bile ducts from the ductus communis with a watery solution of Prussian blue. Harden in Müller and spirit. Cut in gum and mount in balsam.

18. Pancreas of dog, cat, rabbit, etc.—(See "Salivary glands," page 343).

19. Injected pancreas.—Inject the pancreas from the aorta. Harden in Müller and spirit. Cut in gum, and mount in balsam.

CHAPTER XIII.

THE KIDNEY, URETER, AND BLADDER.

THE KIDNEY.

General Characters.—The kidney is a compound tubular gland. It differs from those already considered, however, in the extraordinary length of its tubules, the variations in the nature of the epithelium lining them in different parts of their course, and in the general complexity of arrangement. It is invested with a fibrous *capsule*, which at the hilum joins the connective tissue sheath of the blood-vessels and duct. But the duct in this case as it reaches, or more properly speaking, leaves the organ, is very much dilated, so much so that the usual simple arrangement of parts at the hilum of a gland is somewhat masked.

If a longitudinal section of the kidney passing through the duct or ureter be made (*Fig. 130*), it will be seen to present a characteristic outline which has supplied us with the term *reniform*, or kidney-shaped. The greater part of the surface is convex. At the hilum there is a smaller, well-marked concavity, which is termed the *sinus*, the periphery of which is joined by the expanded end or *pelvis* of the ureter.

The kidney is composed essentially of a series of wedge-shaped lobules, the bases of which are directed to the convex surface, and the apices of which project, in the form of papillæ, into the pelvis of the ureter. This pelvis is lodged in the large hollow or sinus, entrance to which is gained by the hilum. When the sinus of the kidney is laid open, the pelvis of the ureter is observed to break up into two or three—usually two—*diverticula* or *infundibula*. These infundibula are moulded against the projecting papillary ends of the wedge-shaped lobules, which so depress them at various points as to give rise to little cup-shaped hollows. These cup-shaped hollows thus

receiving the ends of the pyramids are termed the *calyces*, and are about twelve in number in man.

The kidney in its embryonic condition is composed of lobes, which consist of a medullary portion covered at its base, and partly at its sides by cortex. The lobes are fused together at their outer part, but the external surface of the organ dips slightly at the line of junction, so that to the naked eye the lobulation is quite distinct. This condition is found permanently in the kidney of the ox and some other animals. In the human kidney, in the course of development, nearly all the cortex comes to lie external to the medullary part, and the surface division into lobes entirely disappears.

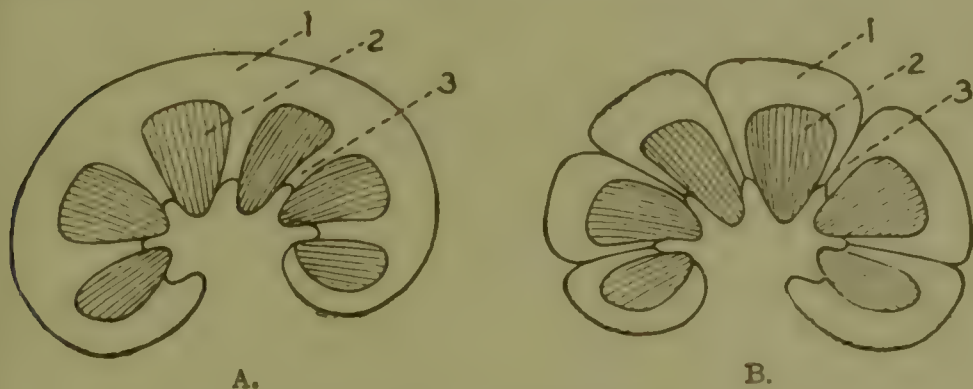


Fig. 1.—Diagram of Kidney, showing development from separate lobules.
A.—Adult kidney, with lobules fused, and external indications obliterated.
B.—Embryo kidney, with lobules still distinct from each other at their periphery.
1.—Cortex. 2.—Medulla. 3.—Column of Bertini.

The substance of the kidney is divided into a cortical and a medullary portion. The *cortex*, to the naked eye deep red in colour and granular, is narrower than the *medulla*, and consists of the fused bases of the wedge-shaped lobules. It presents thus a uniform appearance, as opposed to the medulla, which is represented by the separated apices. The medulla consists of the narrow end of the wedges, which are completely free from each other where they project into the calyces. Outside this apical region, however, they lie side by side, separated from each other by the larger vessels, with their accompanying connective tissue, and also by prolongations downwards of the cortical substance—the columns of Bertini.

These medullary portions of the wedge-shaped lobules are lighter in colour than the cortex, and are not granular but striated. They are termed the Malpighian pyramids. But the

medulla is capable of division into two zones itself—the papillary and the boundary—the latter of which is next to the cortex. The zones vary in breadth in different animals. Roughly speaking, the medulla is broader than the cortex. In the medulla itself the boundary zone is broader than the papillary.

If the cortex be examined with a hand lens it is easy to make out radiating bundles passing into it from the bases of the pyramids of Malpighi. These bundles are continuations of some of the tubules or ducts of which the medulla is composed. They decrease in thickness as they pass towards the periphery of the cortex, and cease in points at an appreciable distance from the capsule. Several of these bundles spring from the base of one medullary segment, and they are termed the *pyramids of Ferrein* (*f*) or *medullary rays*. The cortex between them—the *inter-pyramidal cortex*, as it is termed—is distinctly granular in appearance, and dotted over with small round bodies—the *Malpighian corpuscles*. The inter-pyramidal cortex represents the chief secreting portion of the gland, the medullary rays and Malpighian pyramids the conducting portion.

Connective Tissue Framework of the Kidney.—The connective tissue of the kidney is, on the whole, very small in quantity. The capsule has the peculiarity of being easily separable from the organ, because it sends no well-marked trabeculæ into the interior. It is only united to the kidney by very delicate connective tissue, continuous with that existing in minute quantity between the tubes.

This union, though slight, is not unimportant. The capillary network between the tubules is connected with those of the capsule, and these in turn with the capillaries of the surrounding fat. In chronic cirrhosis of the kidney, too, the capsule becomes adherent to the organ, because the delicate connective tissue, normally uniting the two, shares in the general hypertrophy of this element throughout the gland, and binds them firmly together.

At the margin of the pelvis the connective tissue of the capsule and that surrounding the expanded end of the ureter is continued into the kidney between the pyramids of Malpighi, where it surrounds the large vessels and is again continuous with the small amount of connective tissue between the tubules. In the papillary region there is a little more inter-tubular connective tissue than elsewhere, and in this region the basement membrane surrounding the tubules is fused with it. Throughout the rest of the organ the basement membrane is

readily distinguished as a well-marked line with nuclei occurring in it here and there. It is formed of a condensation of the connective tissue, *i.e.*, of flattened, expanded fibres, with flattened connective tissue cells in the interspaces.

The Tubules and Blood-vessels of the Kidney.—(*Figs. 127, 128, 129*). The uriniferous tubules commence in the inter-pyramidal cortex, *i.e.*, the cortex lying between the pyramids of Ferrein. The terminal, blind end of a tube (*Fig. 128, a*), is expanded and lined by flattened epithelial cells. Furthermore, it is invaginated; and surrounds a tuft of capillary vessels called a *glomerulus*. The glomerulus is completely surrounded by the invaginated end of the tube, except where it joins its stalk, consisting of the afferent and efferent blood-vessels in connection with the capillary tuft (*Fig. 127, 2 and 3*). The outer layer of the invaginated cup rests upon a distinct basement membrane, continuous with the basement membrane of the rest of the tube. This outer layer of epithelium is composed of simple squamous, nucleated cells, and, together with the basement membrane, constitutes the *Malpighian* or *Bowman's capsule*. The inner layer of the cup, formed also of flattened cells, continuous with those of the outer layer around the stalk of the glomerulus, is closely applied to the outer side of the capillary tuft, dipping down into it along the sides of the capillary loops; so that it cannot be distinguished as a separate structure, but is merely recognisable from the presence of its nuclei. There is no distinct basement membrane between this epithelium and the capillary tuft. Between the inner layer of the invaginated cup closely applied to the capillaries, and the outer, which forms, with the basement membrane, the Malpighian capsule, there is a distinct space continuous with the lumen of the first convoluted tubule. The space varies in size with the degree of distension of the capillary vessels.

The Malpighian capsule is connected with the first convoluted tubule (*Fig. 128, b*), of which it is part of the expanded extremity, by a narrow *neck* with a very small lumen, lined by low cubical cells. The neck is very short, and rapidly expands to form the *first convoluted tube*. This, as its name implies, is distinguished by its contorted or convoluted character. It is one of the broadest parts of the uriniferous tube, and belongs to the distinctly secretory part of it. It is lined by a layer of large, roughly cubical cells, whose inner, irregular or conical ends

FIG. 128.

DIAGRAMMATIC REPRESENTATION OF A TUBULE OF THE KIDNEY,
FROM ITS COMMENCEMENT ROUND A GLOMERULUS, TO ITS TER-
MINATION IN THE PELVIS.

- a.*—Cup surrounding glomerulus.
- b.*—First convoluted tubule.
- b.¹*—Spiral tubule.
- c.*—Descending limb of Henle.
- d.*—Loop of Henle.
- e.*—Broad ascending limb of Henle.
- f.*—Irregular tubule.
- g.*—Second convoluted tubule.
- h.*—Junctional tubule.
- i.*—Straight collecting and discharging tubules.
- j.*—Epithelium of pelvis.
- k.*—Sinus of kidney.

FIG. 129.

DIAGRAMMATIC REPRESENTATION OF CIRCULATION OF KIDNEY.

- a.*—Arterial arcade.
- b.*—Interlobular artery.
- c.*—Interlobular vein.
- d.*—Glomerulus.
- v.*—Venous arcade.
- e.*—Afferent arteriole.
- g.¹*—Arteriolæ rectæ coming from arterial arcade.
- g.¹¹*—Venulæ rectæ joining venous arcade.
- h.*—Capillary network between vasa recta.
- i.*—Venæ stellatæ.
- f.¹*—Efferent vessel breaking up into network of capillaries between convoluted tubules.
- f.¹¹*—Efferent vessel passing into medulla as arteriolæ rectæ (*g*).

Fig. 128.

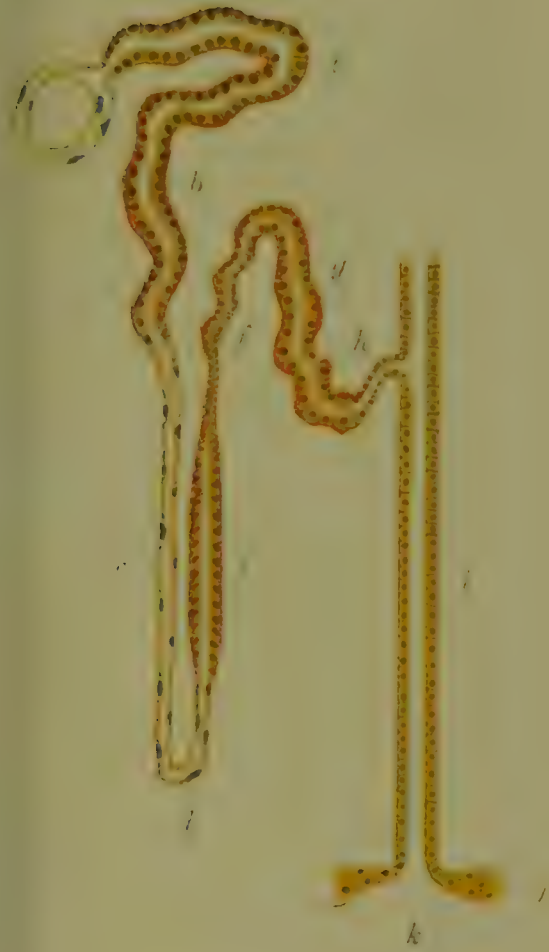


Fig. 129.



project into the lumen of the tube. In cross sections of the latter (*Fig. 131*) the cells frequently appear wedge-shaped, the base of the wedge resting on the basement membrane. The lumen is irregular from the projections of the cells into it, and varies in size, being sometimes considerable, and sometimes little but a stellate fissure. The cells are granular, possess a centrally placed, round nucleus, and are distinctly striated in their outer part.

The first convoluted tubule after repeated bendings upon itself, is continued into the *spiral tubule* (*Fig. 128, b¹*). This is very similar to it in structure, but differs from it in being straight, except for the spiral twist upon it, from which it derives its name. It lies too, not in the inter-pyramidal cortex, but in a pyramid of Ferrein (medullary ray). Its epithelium is a little lower than that of the foregoing, and its lumen consequently wider; the cells, too, are not so distinctly "rodded" in their outer part. The spiral tubule passes down the pyramid of Ferrein, and at the commencement of the medulla terminates in the *descending limb of Henle's tubule* (*c*), which passes through the boundary zone and penetrates the papillary zone for a little distance. It is the narrowest part of the uriniferous tubule, and is lined by flattened cells, which are supported by a distinct basement membrane. The cells are clear, not granular, and possess a nucleus, which causes them to bulge in the centre. When a tubule is seen in longitudinal section, the lumen is shown to be not straight but sinuous, in contrast to the external contour of the tube. This is due to the fact that the cells are so arranged that the nucleated centres of the cells on one side of the tube are on a level with the thin peripheral parts of the cells on the opposite side. Entering the papillary region, the tubule very soon bends backwards upon itself to the periphery again, and this bend is termed *Henle's loop* (*d*). It has now become the *ascending limb of Henle's tubule* (*e*), and as such traverses the boundary layer of the medulla parallel with its former course. As long as it is in the papillary region, it is of the structure already described, but it changes in character as soon as the lower limit of the boundary layer is reached; or it might be more correct to say that the line of change defines the lower limit of the boundary layer. Here it becomes broader, though not so broad as the convoluted tubes, and is lined by deeply-stained striated cells, with round or oval nuclei. The

cells are columnar in shape, and frequently overlap each other in an imbricated manner, that is to say, are not placed vertically upon the basement membrane, but with a slant in one direction. These characteristics are particularly obvious in the ascending limb of Henle's tubule in the dog, but not so distinct in the human kidney.

The ascending limb passes on from the boundary layer of the medulla into the medullary ray of the cortex, which it leaves as the *irregular tubule* (*f*), which lies amongst the convoluted, in the inter-pyramidal cortex. In structure it much resembles the ascending limb, but pursues a zigzag course, and its external contour shows many sharp angles. The lumen of both the ascending limb and the irregular tubule is small. The irregular tubule is also particularly well seen in the kidney of the dog. It terminates in the *second convoluted tube* (*g*), the structure of which resembles precisely that of the first. The second convoluted terminates in a narrow, short *junctional tubule* (*h*), lined by low cubical cells, which passes into a medullary ray to join one of

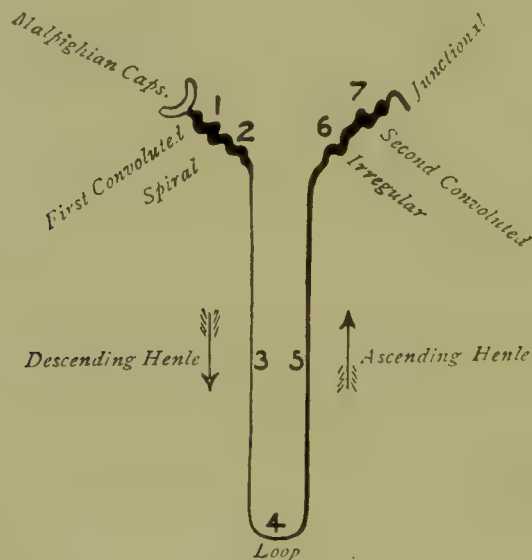


Fig. K.—Diagram of Tubule of Kidney, from capsule of Bowman to junctional tubule.

Fig. K may be of some assistance in enabling the student to fix in his mind the order in which the different parts of a tubule come. The tubule is shown in its entire length with the exception of the straight collecting and discharging portions. It is represented as a test-tube with a funnel-shaped opening, and it will be observed that the two sides of the funnel correspond closely with each other, and the descending with the ascending part of the tube. The drawing is, of course, in no way *ad naturam*, and is only given for mnemonic purposes.

the *straight collecting tubes* (*i*). The straight collecting tube is lined by clear cubical cells, with a central nucleus, which surround a wide lumen. Several straight collecting tubes, after passing through the boundary layer, join together in the papillary region to form a large *discharging tube*, or *tube of Bellini*; and a number of these, varying from 12 to 20, open into the pelvis about the apex of a papilla. The discharging tubule resembles the collecting, but is larger, and its epithelium more columnar in character. At the point where it opens on the papilla, it becomes continuous with the epithelium lining the pelvis of the ureter, which is transitional in character (*j*).

In the papillary region the basement membrane is fused with the connective tissue between the tubules.

The different parts of a tubule may be thus allocated :—

CORTEX.	Inter-pyramidal Cortex.	{	Malpighian capsule.
			1st and 2nd convoluted tubules.
	Medullary ray or Pyramid of Ferrein	{	Irregular tubule.
			Junctional tubule.
MEDULLA.	Boundary zone.	{	Straight collecting tubule.
			Continuation of ascending limb of Henle.
			Spiral tubule.
	Papillary region	{	Straight collecting tubule.
			Descending limb of Henle.
			Ascending " "
			Straight collecting.
	Papillary region	{	Discharging.
			Henle's loop.

The vascular arrangement in the kidney is as complicated as the tubular. The *renal artery* divides into branches at the hilum, which pass outside the epithelial lining of the pelvis to the points of separation between the pyramids of Malpighi. They then run radially outwards till they reach the outer margin of the medulla, where they form *arterial arches* or *arcades* (*Fig. 129, a*), concentric with this line, which anastomose with each other. From the convexities of these arcades, *radiating* or *inter-lobular* branches are given off (*b*), which pass midway between the pyramids of Ferrein or medullary rays in the cortex towards the periphery, decreasing in calibre as they proceed. From these radiating branches, short *afferent arterioles* (*e*) are derived, one for each glomerulus; so that in section there is a row of glomeruli on each side of the vessel, fed by short branches given off from it. The afferent arteriole breaks up into the *capillary*

tuft of the glomerulus, from which the blood is again collected by the *efferent* vessel which leaves the glomerulus at the point at which the afferent enters it. The short efferent vessel (f^1) now breaks up into a *capillary network* (j), which surrounds the tubules both in the inter-pyramidal cortex and in the pyramids of Ferrein. The network in the former has more or less polygonal meshes, in the latter they are elongated, in a radial direction; in each case, of course, the disposition of the network is conformable with the outlines of the tubules between which it occurs.

From this network the blood is collected by the *interlobular* or *radiating veins* (c), which lie in close relation to the corresponding arteries between the pyramids of Ferrein. The radiate veins join *venous arches* (v) at the boundary of the medulla, which are continued between the Malpighian pyramids in company with the corresponding arteries, and finally unite to form the *renal vein*.

The medulla is supplied with blood by means of *arteriolæ rectæ* (g^1), which arise from the concave side of the arterial arches, and pass radially towards the apex of the pyramid between the tubules, where they form a capillary network with elongated meshes, continuous with the capillary network of the cortex. The blood is returned by corresponding *venulæ rectæ*, which join the venous arcades.

But some of the arteriolæ rectæ (g) have another origin. They are the termination of the efferent vessel of the Malpighian bodies immediately outside the medulla (f^{11}). The venulæ rectæ, however, all terminate in the venous arches.

The terminal twigs of the interlobular arteries end in the capillary network between the tubules at the surface of the kidney. The small veins in this region are often star-shaped, and are called the *venæ stellatæ*. An important though minute anastomosis takes place between the vessels here and those of the capsule, which in turn anastomose with vessels in the extra-peritoneal fat.

THE URETER.

The ureter is lined by a layer of transitional epithelium, continuous with that lining its expanded upper end. The epithelium, which is thrown into longitudinal folds, rests upon a foundation of fine connective tissue, the two together constituting the *mucosa*. The connective tissue becomes looser further

out as the *muscular coat* is approached. The latter consists of an inner longitudinal and an outer circular layer. In the lower part of the tube a third external longitudinal layer makes its appearance. In the upper expanded end of the ureter only the circular fibres persist. Outside these coats of non-striped muscle is a connective tissue investment.

THE BLADDER.

The ureters open into the bladder obliquely. The latter is lined by transitional epithelium continuous with that of the ureters. In the contracted state of the viscus, the epithelial layer with the fine connective tissue immediately beneath it, the *mucosa*, is thrown into folds which disappear when it is distended with fluid. Further out the connective tissue becomes looser and coarser, and in this position may be regarded as constituting the *submucosa*. Outside the submucous coat is the *muscular wall* of the bladder, which may be said to be arranged in three divisions; an internal (thin) longitudinal, a middle, transverse or circular, and an outer longitudinal. These may be regarded as being continuous with the coats of the ureter. Thus the external longitudinal coat of ureter spreads out in the bladder in the immediate neighbourhood of the opening of the ureter to form a thin layer. The middle circular is continuous with the circular coat of the bladder, and the outer longitudinal coat becomes the same in the bladder—the so-called “detrusor urinæ.” The bundles of fibres of non-striped muscle do not, however, run very regularly, and there is a good deal of interlacement. At the commencement of the urethra the circular coat is specially developed to form the sphincter of the bladder.

The **urethra** is lined by a layer of epithelium which is transitional in the prostatic part, columnar in the body of the penis, and stratified squamous at the meatus.

Examine the following sections:—

(1.) *Longitudinal radial section of kidney of small mammal (guinea-pig), stained with hæmatoxylin. B.*

Examine this section with the low power in order to make out the general disposition of the parts. Note first the capsule of the organ covering its convexity, and appearing to cease when the hilum is reached. Note that no trabeculæ pass into the

FIG. 130.

DIAGRAMMATIC REPRESENTATION OF PARTS OF KIDNEY.

- a.*—Capsule of kidney.
- b.*—Ureter.
- c.*—Sinus of kidney.
- d.*—Hollow between calyces (*e*), which receive the,
- e.*—Papillæ of the Malpighian pyramids.
- f.*—Pyramid of Ferrein.
- g.*—Cortical labyrinth between pyramids of Ferrein.
- h.*—Interlobular vessel with branches going to glomeruli.
- i.*—Vascular arcade at junction of medulla and cortex.

FIG. 131.

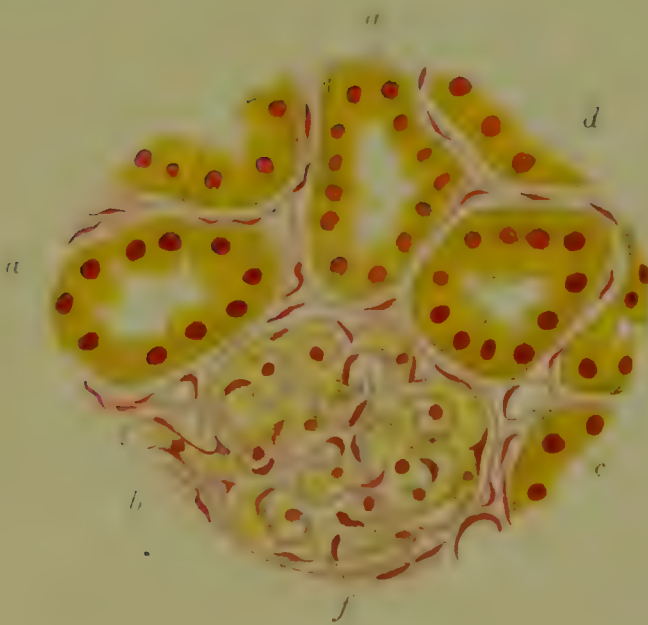
S. KIDNEY, HUMAN (CORTEX), STAINED WITH PICO-CARMINE $\times 300$.

- a.*—Section of convoluted tubule.
- b.*—Glomerulus.
- c.*—Capillary vessel between tubules.
- d.*—Intertubular connective tissue.
- f.*—Capsule of Bowman.

Fig. 130.



Fig. 131.



substance of the kidney, from its under surface. Observe two or three sections of papillæ projecting into the concavity of the sinus. Now distinguish between the cortex and medulla. The cortex is easily recognisable as a broad external layer dotted over with Malpighian bodies. In the medulla there are no Malpighian bodies, and it is seen to consist of a mass of closely-set tubules running radially from the papillæ outwards. If the line of junction of the cortex and medulla be now approached, it will be seen that the Malpighian pyramids are not sharply separated from the cortex, but that their substance is continued into it in the form of radiating bundles—the pyramids of Ferrein. Note that none of the round Malpighian bodies are to be found in these medullary rays; they are limited to the spaces between them, the interpyramidal cortex. Under this power the Malpighian bodies appear to be about the size of a pea, and are dotted over with nuclei (the nuclei of the capillary walls and perhaps of a little connective tissue between the capillaries), stained deeply with the reagent. In many places the glomeruli will be seen to have fallen out, leaving a clear space, bounded by the basement membrane, lined with flattened epithelium. The Malpighian bodies are surrounded by the sections of the convoluted tubules amongst which they lie. Many of the tubes are cut transversely, but a certain number of them show the convolutions quite well. Observe that there is a narrow area beneath the capsule which is free from Malpighian bodies.

At the junction of the cortex and medulla, look for sections of larger vessels, variously cut. Some of them are sections of the arcades, either arterial or venous; others of the vessels before they form the arcades, in which case they will run between the bases of the Malpighian pyramids.

(2.) *Longitudinal radial section of portion of human kidney, stained with picro-carmin or hæmatoxylin. F. or B. (Fig. 131.)*

This is a section through the cortex and medulla, including a whole or part of a Malpighian pyramid. Use it especially for high power examination.

Under the low power identify some of the structures mentioned above: the capsule, with the cortex immediately beneath it, free from Malpighian corpuscles; the inter-pyramidal cortex, composed chiefly of convoluted tubes and Malpighian bodies; one or more pyramids of Ferrein. In the pyramid of Ferrein endeavour to recognise some of the tubules. The spiral tubule is

distinguishable from the collecting by its greater breadth (in this region), and the larger size and granularity of the cells lining it. At the junction of the medulla and cortex, note again the large blood-vessels imbedded in connective tissue. Put on the high power. Find first a Malpighian body in the cortex, and examine its structure (*Fig. 131*). Observe the capsule (*f*) showing as a clear line surrounding the glomerulus, but distinct from it, with the nuclei of its flattened epithelial lining seen here and there. The structure of the glomerular tuft is not always very easily made out. Here and there, portions of the capillary loops (*b*), of which it is composed, may be seen as such, with blood corpuscles in their lumen, their walls indicated by lines with nuclei upon them—the nuclei of the epithelial cells. Sometimes, especially if the tuft is empty and collapsed, nothing but a confused mass of nuclei can be made out, with an occasional indication of a capillary wall. The reflection of epithelium over the glomerulus from the capsule cannot be made out as a separate structure; all that will be seen are the nuclei of its epithelial cells in section. Look for the afferent or efferent vessel. This will not be seen in most of the corpuscles, but it may be in some. Occasionally a Malpighian corpuscle is so cut that the narrow neck connecting it with a convoluted tubule is shown, but this is exceptional. It will readily be understood that in the case of a spherical body with a duct at one pole and a vessel at the other, the chances are much against either of these connections being shown in a chance section. They will be seen in two of the specimens described below.

Surrounding the Malpighian body, observe the sections of the convoluted tubules (*a*) cut in various directions. Note the irregular lumen of one of them. In most specimens the lumen is small, but it may vary very much in size. The irregularity of it is due to the presence of the cells projecting into it. Note the cells themselves. Their outlines are not distinct; they are granular, their nucleus is round, deeply stained, and placed in the centre, and the cells are distinctly striated or “rodded” in their outer part. Look for the nuclei of the basement membrane surrounding the tubules. Between the tubules themselves, note the very small amount of connective tissue. Here and there, a few blood corpuscles in lines may be seen, indicating the presence of the intertubular capillary network. The connective tissue is mainly indicated by the nuclei of its cells.

Amongst the large convoluted tubules of the inter-pyramidal cortex, look for sections of the irregular tubules. They are smaller in diameter, and are easily recognisable from their zigzag outline with projecting angles. They are deeply stained, and distinctly rodded. They are better seen, however, in the kidney of the dog.

Examine a pyramid of Ferrein, and observe in it the large spiral tubules having the same structure as the convoluted, and the collecting tubules lined with clear, low, columnar cells.

The broad and narrow limbs of Henle's tubule in the boundary layer are seen to more advantage in the kidney of the dog, but may be looked for here. The narrow limb presents much the appearance of a longitudinally cut capillary vessel.

In the papillary region of the Malpighian pyramid, note especially the collecting tubules cut longitudinally. Note their width, their large lumen, and lining of clear columnar cells. Observe that in this region they are considerably larger than their commencement in the pyramids of Ferrein. Examine the surface of the papilla for the transitional epithelium lining the pelvis. Occasionally its continuity with the epithelium of a discharging tubule may be demonstrated.

(3.) *T. S. Papillary region of a Malpighian pyramid of human kidney, stained with picro-carmin or hæmatoxylin. F. or B. (Fig. 133.)*

This is a section which shows transverse sections of Henle's loop, consequently it is taken at a level, just below the inner margin of the boundary layer. Under the high power note the transverse sections of the collecting tubes, and it may be of discharging ones (*a*). These collecting and discharging tubes vary very much in size, as may be seen in the figure. Between them note the increased amount of connective tissue (*d*) which supports many smaller tubes cut transversely. Some of these are sections of capillary blood-vessels (*c*), others of the narrow part of Henle's tubule (*b*). Note the difference between these two. The capillary sometimes contains blood, which at once differentiates it; but it may be recognised also by being somewhat smaller, and by the cells of its walls being thinner and not bulged inwards in the centre, to the same extent as is the case with the cells of the tubules, and by the cell substance being still clearer. Where the columnar cells, lining a collecting tube, have fallen out—and this is a common occurrence in thin sections cut in gum—note the

FIG. 132.

L.S. BOUNDARY LAYER, KIDNEY OF DOG, STAINED WITH HÆMATOXYLIN
× 300.

- a.*—Broad ascending limb of Henle's tube.
- b.*—Straight collecting tubule.
- c.*—Henle's loop.
- d.*—Capillary blood-vessels.
- e.*—Connective tissue.

FIG. 133.

T.S. PAPILLARY REGION, HUMAN KIDNEY, STAINED WITH HÆMATOXY-
LIN × 300.

- a.*—T.S. Collecting tubes.
- b.*—T.S. Henle's loop.
- c.*—Capillary blood-vessels.
- d.*—Connective tissue.

Fig. 132

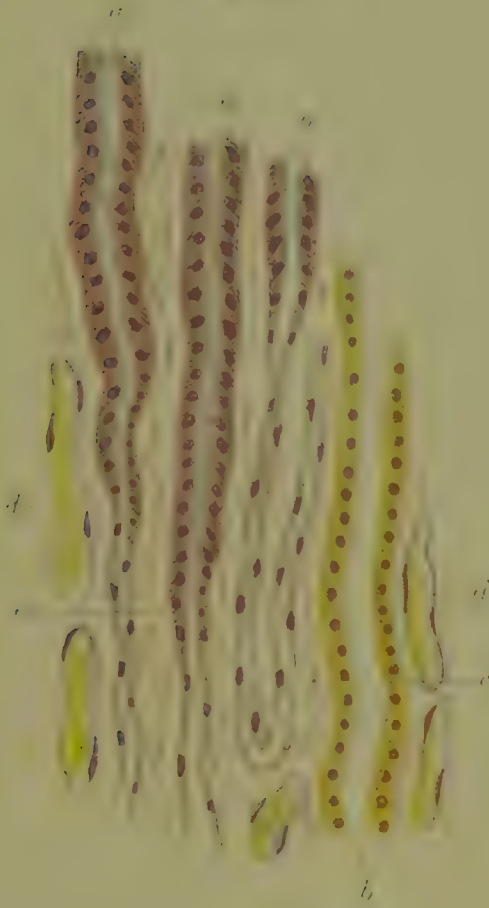
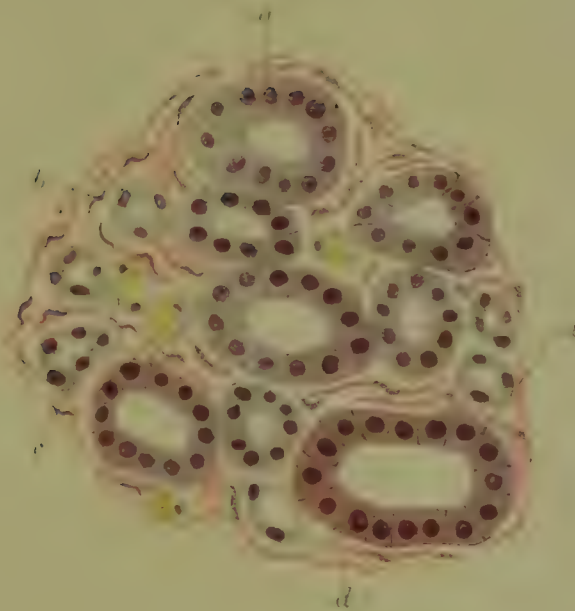


Fig. 133.



clear round space left defined by the basement membrane, which is here fused with the rest of the connective tissue.

(4.) *Longitudinal radial section of Malpighian pyramid and cortex of kidney of dog, stained with hæmatoxylin. B. (Fig. 132.)*

This specimen is of particular service in demonstrating Henle's, and the irregular tubule.

Examine first without a lens, and observe that the outer part of the medulla is here seen as a distinct layer—the boundary zone, which even to the naked eye presents a markedly striated appearance. Look now at this layer with a hand lens, and note that the striated appearance is due to the presence in it of a number of deeply stained tubules placed radially side by side. The tubules can be seen to have a very distinct wave upon them at the cortical end of the medulla, almost a corkscrew appearance, which decreases as the inner part of the boundary layer is reached, where they are almost straight. These are the broad, ascending portions of Henle's tubule.

Put on the low power and confirm these appearances. Trace the tubules up into the pyramids of Ferrein, and observe their continuation there, more deeply stained than the other structures of the pyramid. In this part of its course the tubule is not yet irregular or zigzag, but gives indications here and there of its becoming so. In the interpyramidal cortex look for sections of the zigzag tubules. They are readily recognisable from their shape and the depth of their staining. Trace the broad limbs of Henle in the boundary layer inwards, and note that at the edge of the papillary region they narrow, and appear under this power to terminate somewhat in a point; in reality they join here the narrow part of Henle's tubule.

It may be noted here that the section should not be very deeply stained. If it is so, the convoluted tubes, the spiral, the irregular and the broad part of Henle's tube are all equally affected. If it is only lightly stained, the broad part of Henle, its continuation in the pyramid of Ferrein, and the irregular tubule are picked out very distinctly; that is to say, they take up the stain more quickly than the convoluted tubules do.

Under the high power examine the boundary layer in its lower part (*Fig. 132*). Note the longitudinally cut, deeply stained tubules (*a*), with a small lumen surrounded by rodlike imbricated epithelium. Trace these towards the papilla, and note their ending in the capillary-like narrow limb. Observe the longitudi-

nal sections of this limb particularly, noting the sinuosity of the lumen as already described, the alternate positions of the nuclei of its wall, etc. Here and there observe collecting tubes (*b*), and capillary blood-vessels (*d*). There is more connective tissue here than in the cortex. Examine one of the irregular tubules in the cortex, noting its shape and the rodlike character of its epithelium. In both the broad limb of Henle and here also, the epithelial cells seem to be rodlike throughout their whole depth. Endeavour to trace a connection between a zigzag tubule and the continuations of one of the ascending limbs of Henle in the pyramid of Ferrein. This can usually be done without difficulty, especially where the staining has been favourable to the differentiation of these parts of the uriniferous tubule from the rest.

(5.) *Longitudinal radial section of kidney of small mammal, injected with gelatine mass. Unstained. B.*

Under the low power recognise the sections of large vessels, from some of which the injection may have fallen out, at the junction of the medulla and cortex, and the radiate arteries passing outwards between the pyramids of Ferrein. The connection between the radiate arteries and the arches is not, of course, always to be seen. It depends on how the section has passed. The radiate arteries may be seen giving off short afferent branches (slightly curved with the convexity towards the capsule of the kidney) to end in the glomeruli. A glomerulus with its afferent arteriole has somewhat the appearance, especially in an injected specimen, of a raspberry on its stalk. Endeavour to find one in which the efferent vessel is also shown, leaving it at the point of entrance of the afferent, and breaking up into a capillary network with polygonal meshes between the convoluted tubules. Observe that the pyramids of Ferrein are marked out by the elongation of the capillary meshes in them.

Below the level of the junction of the cortex and medulla, note the leashes of vasa recta passing down into the medulla, where they break up into a capillary network with elongated meshes.

These capillary networks, it will be understood, are continuous with each other throughout the organ, the difference in the shape of the meshes depending merely on the character of the tubules between which they lie.

(6.) *Teased preparation of kidney of guinea-pig (for isolated convoluted tubules and Malpighian bodies).*

In this preparation, which has been previously treated with

hydrochloric or nitric acid, the convoluted tubes are separated from each other, and in many cases show their attachment by a narrow neck to the Malpighian bodies.

(7.) *Longitudinal radial section of embryo kidney (human), stained with hæmatoxylin. B.*

Under the low power note the distinctly separated lobes, each composed of a medullary portion (pyramid of Malpighi) surrounded externally, and, to some extent, at the sides, with a cortical zone. It is as though a cap of cortex had been placed over the broad end of the pyramid—a cap which reached half way down the sides. Note the fusion of the sides of adjacent caps, and that it is often merely a partial one, the connective tissue of the capsule appearing as a line indicating the separation. On either side of this apparent prolongation from the capsule, the Malpighian corpuscles may be seen in various stages of development. These rows of Malpighian bodies constitute the columns of Bertini. Under the high power find one of the dilated extremities of the tubules being invaginated by the capillary tuft to form a Malpighian capsule. Look for instances in which the latter is cut so as to show its connection with a convoluted tubule.

(8.) *T. S. Ureter of man, cat, or dog, stained with picro-carmin, hæmatoxylin, or borax-carmin. F. or B.*

(9.) *V. S. Bladder of cat, stained with picro-carmin, hæmatoxylin, or borax-carmin. F. or B.*

In both these specimens under the low power note the general arrangement of parts; under the high power examine especially the lining of transitional epithelium (page 104, *Fig. 16*).

APPENDIX TO CHAPTER XIII.

METHODS OF PREPARATION.

1. Kidney of small mammal, radial section.—Divide the kidney of a guinea-pig longitudinally or transversely, and radially, and harden in Müller's fluid, or Müller and spirit. Stain in bulk in borax-carmin, and cut in paraffin; or cut in gum, stain in picro-carmin or hæmatoxylin, and mount in Farrant or balsam.

2. L.S. Malpighian pyramid of human kidney.—Obtain the kidney as soon after death as possible, divide it longitudinally and radially, and subdivide each of the halves by incisions vertical to the cut surface between the Malpighian pyramids. The kidney may with advantage be divided still further, the resulting pieces being wedge-shaped, and including portions of both the cortex and medulla. Harden in Müller's fluid, or Müller and spirit; cut in gum; stain in hæmatoxylin, and mount in Farrant in preference to balsam if the section is thin, as the definition is better; or stain in picro-carmin, and mount in Farrant. The wedge-shaped piece, including both cortex and medulla, is rather large to be cut in paraffin, but a part of it may be dealt with in this way, being previously stained in bulk in borax-carmin.

3. T.S. Papilla of Malpighian pyramid (human).—Harden the apical part of a Malpighian pyramid of the human kidney in Müller's fluid, or Müller and spirit, stain in bulk in borax-carmin, and cut in paraffin; or cut in gum, stain in picro-carmin or hæmatoxylin, and mount in Farrant or balsam. Transverse sections of **pyramids of Ferrein** and **boundary zone** may also be prepared in the same way.

4. L.S. Malpighian pyramid of dog's kidney (for broad part of loop of Henle and irregular tubules).—Cut in same way as No. 2. Harden in Müller, or Müller and spirit; cut in gum, stain in hæmatoxylin, and mount in balsam.

5. Injected kidney of mammal.—If the animal is a small one, *e.g.*, rabbit or guinea-pig, inject with gelatine mass from the aorta; a dog, on the other hand, may advantageously be injected from the renal artery. Keep the pressure low. Harden in Müller, or Müller and spirit; cut in gum, and mount in balsam.

6. Isolated tubules of the kidney.—Place small cubes ($\frac{1}{8}$ in.) of cortex of kidney of guinea-pig in ordinary hydrochloric acid for three to four hours, or in 40 per cent. solution of nitric acid for two to four hours. Wash in water, and leave them there for eighteen to twenty-four hours. The pieces swell

up, and fragments may be gently pressed in a drop of water between the slide and the cover-glass, when the tubules readily separate from each other (Stirling). Look especially for the connection between the convoluted tubule and Bowman's capsule. Other parts of the organ treated in the same way afford demonstration of their constituent tubules in an isolated condition.

7. Fœtal kidney.—Obtain the lobulated kidney of a human fœtus. Harden in Müller, or Müller and spirit; cut radial sections in gum, stain in picro-carmin or hæmatoxylin, and mount in Farrant or balsam; or stain in bulk in borax-carmin, and cut in paraffin. The kidney must be *fresh*, and must be incised before being placed in the hardening fluid.

8. T.S. Ureter.—A human ureter will do if it can be obtained fresh; if not, that of a cat or dog. Harden in Müller and spirit, stain in bulk in borax-carmin, and cut in paraffin; or the sections may be cut in gum, and stained in the usual manner subsequently. If it be desired to show the epithelial lining rather than the general structure, harden in Flemming's fluid, stain in bulk, and cut in paraffin.

9. Bladder of cat.—It is advisable on the whole to use the contracted bladder. Remove the bladder of a cat recently killed, divide it into two parts with a sharp razor, and place them in an excess of Müller's fluid, ammonium bichromate 2 per cent. solution, or Müller's fluid and spirit. If rapid hardening be desired (and this is an advantage here as the epithelium is very liable to injury) chromic acid and spirit may be used, or Flemming's solution. Complete the hardening in spirit, stain in bulk in borax-carmin, and cut in paraffin. It requires care to get a good specimen of the epithelial lining.

CHAPTER XIV.

THE SKIN, EPIDERMIC APPENDAGES, AND
TERMINATIONS OF SENSORY NERVES.

THE SKIN.

THE skin consists of an epiblastic portion, the *epidermis*, and a mesoblastic, the *dermis* or *cutis vera*.

The epidermis, formed of stratified squamous epithelium, shows two main divisions ; a superficial one, the *stratum corneum* which varies very much in thickness in different parts of the body ; and a deep one, more uniform in character, the *stratum Malpighii*. Between these two main layers are two narrower ones, the *stratum granulosum* and *stratum lucidum*.

The dermis or true skin may also be considered in two divisions, though these are not sharply divided from each other ; a superficial one of fine, closely-set, connective tissue immediately beneath the epidermis, with which it is closely adherent ; and a deeper, looser layer corresponding to the submucosa of a mucous membrane. Between the deeper part of the dermis and the subcutaneous connective tissue, free movement of the skin on the subjacent parts can take place, comparable with the movement of the mucosa of the stomach on its muscular coat.

The **epidermis**.—The *stratum Malpighii* rests upon the surface of the dermis, which is condensed to serve the purpose of a basement membrane. The surface of the dermis is not, however, a flat one, but is raised into numerous conical papillæ, some of which are simple, while others are divided or compound.

Between the papillæ of connective tissue, the epithelium dips down, so that the epidermis exactly fits the surface of the cutis vera. The *stratum Malpighii* consists of several layers of

epithelial cells, which differ in shape and size at different depths. The lowest layer of all, that immediately in contact with the basement membrane, consists of a layer of granular, somewhat columnar-shaped cells, placed vertically side by side. They have a relatively large oval nucleus, which is frequently seen undergoing division. This is the germinal layer of cells from which those in the planes above have been produced.

The cells of the main mass of the Malpighian layer are larger, polygonal in shape, and possess a centrally placed spherical nucleus. They are not so granular, and do not stain so deeply as those of the germinal layer. Their relations to each other are peculiar in that they are not cemented together by (in section) a straight margin, but by means of short prick-like processes which beset each cell like the spines of a horse-chestnut. The prickles of adjacent cells do not interlock, but meet each other end to end, so that the main body of each cell is incompletely isolated from its neighbours, by a number of fine channels—the spaces between the prickles. The prickles themselves, by which the cells are brought into contact with each other, are termed inter-cellular bridges; the spaces or channels between them allow of the free percolation of lymph throughout the layer, which is necessary for its nutrition. In consequence of their peculiarity, the cells are termed *prickle-cells*.

The cells of the Malpighian stratum throughout its main mass are, as stated, of this character, viz., polygonal, and surrounded with spines, but they show a certain amount of deviation from it, both in the immediate neighbourhood of the germinal layer, and also beneath the stratum granulosum. There is no distinct line of demarcation between the germinal layer and the cells immediately above it, the transition in size and shape, from the small columnar cell to the large polygonal one, being more or less gradual. The cells immediately beneath the stratum granulosum begin to lose their polygonal shape, and tend to become slightly flattened horizontally.

The *stratum granulosum* consists of one or two layers of cells which differ from those of the stratum Malpighii, in being flattened, or lozenge shaped, in being destitute of prickles, and in exhibiting the granules in their protoplasm, from which the layer derives its name. The granules are said to be composed of eleidin, which is, ultimately, to become the keratin of the cells of the stratum corneum. The cells of the stratum

granulosum stain deeply with hæmatoxylin, carmine, osmic acid, and most reagents.

The *stratum lucidum*, also a narrow layer, is composed of a few strata of flattened cells, apparently fused together. They constitute a homogeneous translucent band, which may often be stained deeply with picric acid. Flattened nuclei, appearing rod-shaped in section, are sometimes to be seen in it here and there.

These two layers, and the surface of the stratum Malpighii, do not follow the line of the prolongations of the latter between the connective tissue papillæ. They are flat as the stratum corneum outside them is, except for the slight depressions corresponding with the furrows seen on the surface with the naked eye.

The *stratum corneum*, to which the variations in thickness of the epidermis in different parts of the body are mainly due, consists of many layers of flattened scales or plates, composed of keratin. The nuclei of the cells are sometimes still to be seen in the deeper layers, but in the superficial the conversion to keratin is usually complete. These scaly plate-like cells of the stratum corneum are being constantly worn off at the surface, and as constantly replaced by others from below. Division takes place for the most part in the germinal layer, but to a small extent also in the rest of the stratum Malpighii. The stratum corneum stains readily with picric acid, but not so readily with hæmatoxylin and carmine.

The **dermis, cutis vera, or true skin.**—This is composed of ordinary connective tissue—white and elastic fibres and connective tissue cells. Immediately beneath the epithelium, where it forms the papillæ, the fibres are fine and closely arranged; in the deeper parts it is more areolar in nature, with large meshes bounded by coarser bundles of fibrous tissue.

The dermis contains blood-vessels, lymphatics, and nerves, with their terminations. The larger branches of the blood-vessels are found in the deeper part. Immediately beneath the epithelium is a fine capillary network.

The nerve terminations connected with the sense of touch are Wagner's corpuscles, found in the papillæ; the Pacinian bodies in the deeper part of the dermis, or in the subcutaneous connective tissue; and the nerve fibrillæ terminating amongst the cells of the stratum Malpighii.

Clusters of fat cells occur in the deeper part of the dermis and in the subcutaneous tissue, and when in large quantity, form a well-marked layer of adipose tissue.

But in addition to the structures already named, two others of epidermic origin are found, namely, the sweat glands, and the hair follicles.

The sweat glands vary in number and size in different parts of the body. They are most numerous in the skin of the palms of the hands and the soles of the feet.

A sweat gland is a simple tubular gland, consisting of a duct and a secreting portion, the whole being a down growth of the surface epithelium. The secreting portion and the lower part of the long duct is very much coiled upon itself, and lies in the deeper part of the dermis.

The duct is narrower than the secreting part of the tube. It has a narrow, regular lumen, surrounded by two layers of somewhat cubical nucleated cells, which rest on a basement membrane, continuous with the surface condensation of the rest of the cutis vera. The duct joins the epidermis between two papillæ, *i.e.*, at the apex of one of the downward prolongations of epithelium with which it is continuous. Its cells fall into series with those of the stratum Malpighii, and its lumen is continued upwards through the successive layers of the epidermis to open on the surface. In its passage through the epidermis it takes not a straight but a corkscrew course, and increases in diameter as it reaches its termination. The duct stains more deeply than does the secretory part of the tube. The secretory portion is broader than the duct, and is lined by a single layer of larger and more irregularly shaped cells. Its lumen also is wider, and at the same time irregular, as seen in *Fig. 136, f.* The cells, which are granular, nucleated, and stain less deeply with reagents than do those of the duct, rest on a similar basement membrane. But we have to note here the presence of non-striped muscle fibres, which run longitudinally or spirally between the epithelium and the basement membrane. They are placed singly at regular intervals from each other (*d*), so that they by no means form a continuous layer. Their function is, presumably, associated with the expulsion of secretion from the tube.

THE EPIDERMIC APPENDAGES.

The Hair Follicles.—"A hair is a development, in the form of a cylinder, of a cap of corneous epidermis surmounting a papilla of the dermis, sunk to the bottom of a tubular pit or involution of the skin called a hair follicle."—(Foster.)

The body, generally, except in certain regions, such as the palms of the hands and the soles of the feet, is covered with hair of a fine character—the *lanugo*; but the structure of a hair follicle, as about to be described, is only to be made out in sections of parts of the skin, such as the scalp, where the hairs are of considerable size.

The following are the parts of a hair follicle from without inwards :—

	(a,) Fibrous investment and basement membrane.	
Epithelial	(b,) Outer root sheath.	} Henle's layer.
	(c,) Inner root sheath.	
	(d,) Hair.	} Huxley's layer.

The *fibrous investment* (*Fig. 140, a, b*) is continuous on the one hand with the superficial dermis beneath the epithelium of the skin, and on the other with the connective tissue papilla at the root of the hair. It is arranged in two layers, an outer longitudinal and an inner circular, the latter of which supports a capillary network. Between the fibrous investment and the hair follicle proper is a very distinct basement membrane, continuous with the surface condensation of the dermis elsewhere. It is bright and refractile, with a double contour, and can be traced below from one side of the follicle to the other, over the *dermic papilla*. It is termed the *hyaline membrane*.

The *outer root sheath*, or Malpighian layer (*d*), is continuous above with the stratum Malpighii, which it closely resembles in structure, except that the cells have no prickles. It consists in its broader part (midway in the length of the follicle) of three, four, or more layers of cells, but narrows considerably as it approaches the bulb—the expanded lower end of the follicle surrounding the papilla. Its external layer of cells is continuous with the germinal cells of the Malpighian layer of the skin.

The *inner root sheath* (*e, f*), narrower than the outer, consists of two layers of cells (Henle's and Huxley's) which are, however, fused to form one homogeneous layer in their upper part. As

the bulb is neared, the two layers become differentiated. The outer of these—Henle's—is narrow, homogeneous, and refractile in appearance, and is composed of squames, arranged with their flat surface towards the axis of the hair. Nuclei, rod-shaped in section, may sometimes be made out, and more rarely the outlines of the individual cells. The inner—Huxley's—consists of somewhat polygonal small cells, arranged two or three deep, with distinct, often quadrangular nuclei. The layer stains deeply with reagents, especially hæmatoxylin, and thus stands out by contrast with Henle's layer and the hair itself. Within Huxley's layer is found the hair itself.

The **hair** is cylindrical in shape, and possesses (1,) a cuticle ; (2,) a cortex ; (3,) (sometimes) a medulla.

The *cuticle* consists of a thin covering of imbricated, flattened, non-nucleated scales, which overlap each other from below upwards. The *cortex*, or main mass of the hair, is composed of fibrous non-nucleated cells, which have undergone the keratin transformation. They are arranged longitudinally, and closely cemented together. The cortex has the appearance of being striated longitudinally. Pigment granules are sometimes found in lines between the cells. The *medulla*, when it exists, consists of a central row of quadrangular cells, which have become hollowed out in their interior, and appear black from the presence of air in them.

The above is a general account of the structure of the layers of a hair follicle with the hair inside it, but it is necessary to consider further the arrangement of parts at different levels of the follicle.

A little below the level of the general epidermis, the *sebaceous glands*, outgrowths from the outer root sheath of the follicle, open into its neck. The sebaceous glands are saccular ; they have a small short duct into which the alveoli open without the intervention of secondary ducts. The basement membrane surrounding the alveoli is continuous with the hyaline membrane of the hair follicle. The alveoli are lined externally with a layer of cubical cells, continuous with the outer layer of the outer root sheath. Within these are cells more polygonal in character ; and in the centre of an alveolus are cells which have undergone fatty degeneration, and are breaking down to form the sebum, or secretion of the gland, which is poured through the mouth of the hair follicle upon the surface of the skin. The

FIG. 134.

V.S. HUMAN SKIN, FROM PALMAR SURFACE OF FINGER, STAINED
WITH HÆMATOXYLIN $\times 30$.

- a.*—Stratum corneum of epidermis.
- b.*— „ lucidum „
- c.*— „ Malpighii „
- d.*—Sweat ducts.
- e.*—Sweat glands and ducts
- f.*—Fat cells.
- g.*—Pacinian corpuscles.

FIG. 135.

V.S. HUMAN SKIN, STAINED WITH HÆMATOXYLIN $\times 300$.

A.—Epidermis.

B.—Dermis, or cutis vera.

- a.*—Stratum corneum.
- b.*— „ lucidum.
- c.*— „ granulosum.
- d.*— „ Malpighii.
- e.*—Wagner's corpuscle in papilla of dermis.
- f.*—Nerve fibre terminating in Wagner's corpuscle.

Fig. 134.

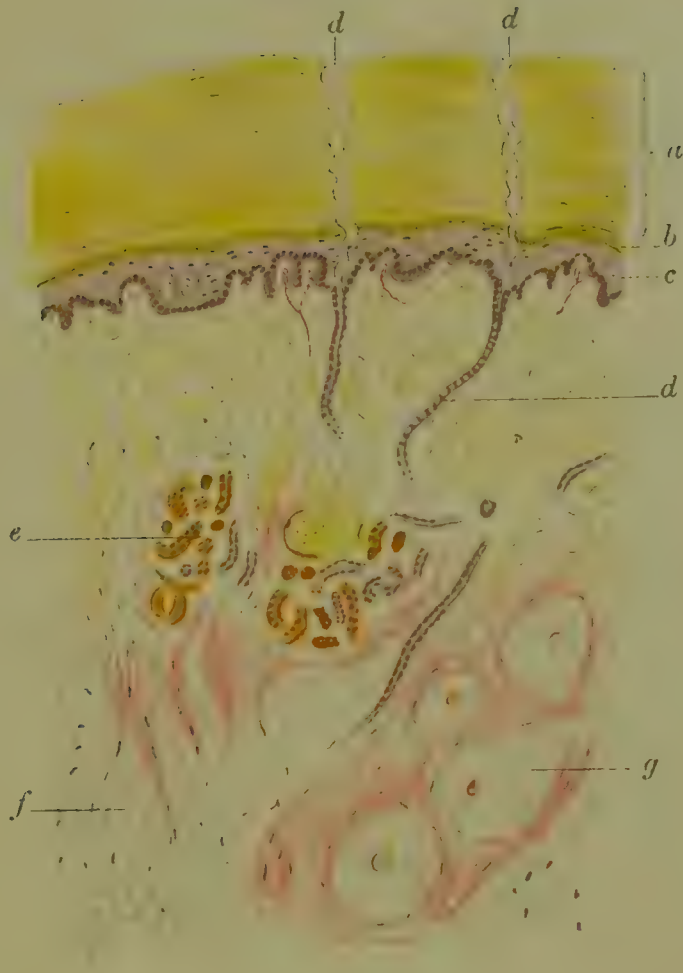


Fig. 135.



glands are, in fact, solid outgrowths of the Malpighian layer, the central cells of which break down to form the secretion, the supply being renewed by proliferation of those at the periphery. The neck of the hair follicle thus indicated by the point of entrance of the ducts of the sebaceous glands is of importance from a descriptive point of view. It is just above this point that the corneal layer and stratum lucidum of the skin, after dipping down from the surface along with the Malpighian layer, cease; it is just below this point that the inner root sheath commences. Here, and for a considerable distance downwards, the inner root sheath is homogeneous, and apparently translucent. Passing downwards from the neck, the follicle broadens considerably, due to the increased thickness of the Malpighian layer. It then narrows again towards the bulb, where it broadens for a second time, the bulb being the broadest part of the follicle.

The arrangement of parts in this region is a little complicated. It is shown in *Fig. 140*. The stratum Malpighii becomes narrower as it reaches the bulb, and may be represented by only one layer of cells. These, when traced still further, become continuous with a single layer of cubical or columnar cells immediately above the papilla. This layer is, in fact, the continuation of the germinal or outer layer of the Malpighian stratum, and lies immediately within the basement membrane.

The hair itself, when traced towards the bulb, gradually increases in diameter, and at the bulb itself expands with more or less suddenness, so that it becomes continuous with the greater part of the two limbs (in section) of the bulb, which embrace the papilla. This expanded portion of the hair, by its outer margin, comes almost into contact with the Malpighian layer in its narrowest part, there being space only left between them for the narrowed prolongations of Henle's and Huxley's layers to pass into the bulb. For some little distance above the bulb Henle's and Huxley's layers are distinct from each other, and immediately above the bulb, in the concavity formed by the hair and its expansion, they are at their thickest. The cuticle of the hair itself is in this region very plainly seen, as the cells have not yet become flattened so as to form scales. They are seen (in section) as tall columnar cells, possessing a well marked, oval nucleus, and placed with a slant upon the shoulder of the root of the hair, so that they overlap each other from below upward.

The bulb of the hair follicle is composed of polygonal cells, in which the several layers of the hair follicle and the hair itself become lost, at about the level of its middle.

The hair itself is undoubtedly produced from the germinal cells immediately covering the papillæ. Between these and the fibre cells of the hair there is a gradual transition. When the hair possesses a central medulla, this is due to a growth in its axis of cells continuous with the germinal layer, which remain as a separate core.

The dermic papilla, continuous with the dermic investment, is composed of fine connective tissue, and supports a capillary network. The shape of the papilla varies, but is expanded in its upper part, and joins the root of the dermis by a constricted neck. In the large tactile hairs of animals it is not uncommon for the connective tissue of the papilla to be prolonged at its summit for some distance, as a core to the hair itself, as shown in *Fig. 140*; it may, on the other hand, be rounded off, and not penetrate the hair, as shown in *Fig. 139*.

The hair follicles are supplied with nerves similarly to the rest of the epidermis. The ultimate fibrillæ into which the axis cylinder of the nerve fibre splits, penetrate between the cells of the Malpighian layer, from the dermic covering and from the papilla of the bulb.

The hair follicles are not placed vertically, but obliquely, consequently a section to cut them longitudinally requires to be made in a special plane. The *erector pili muscle*, a well marked band of non-striated fibres, is placed in the obtuse angle formed between the hair follicle and the epidermis (*Fig. 142, c*). It is attached on the one hand to the basement membrane beneath the epithelium, and on the other to the hyaline membrane a little above the bulb. The sebaceous gland of that side, usually the largest, is included in the triangle thus formed, and causes the erector to bulge slightly outwards at the point at which it comes in contact with it. The muscle, by its contraction, causes the hair to "stand on end." It is purely involuntary in man, but appears to be under the influence of the will in some animals.

The large tactile hairs of animals (*Fig. 139*), are remarkable not only from their size, but from the presence of a blood sinus between the two layers of the dermic covering, which are here specially developed. The sebaceous glands are merely rudimentary. The blood sinus is traversed by trabeculæ of connective

tissue, which form a network, on the strands of which epithelial plates, continuous with those lining the wall of the sinus, occur.

Development of the hairs.—A hair commences as a down-growth of the epithelium into the connective tissue beneath. The hyaline membrane is early distinguishable, and is continuous with the basement membrane beneath the surface epidermis. The down-growth increases in size at its lower extremity, and like the enamel germ of a developing tooth becomes invaginated from below by a dermic papilla. The hair itself and the internal root sheath are developed from the epithelial cells immediately covering the papilla; they push their way amongst the original cells of the down-growth (which fall away on either side of the advancing core, and remain as the outer root sheath) till the surface is reached. The hair continues to grow during life by a proliferation of the cells above the papilla. The sebaceous glands are developed as solid outgrowths from the outer root sheath at the neck of the follicle, the central cells of the alveoli degenerating to form the sebum. Some of the cells of the original down-growth in the path of the growing hair also seem to undergo the degeneration independently. When hairs are shed during life they are replaced by a new development in the hair follicles, from which they are cast out. The old papilla and bulb appear to atrophy, the new bulb being formed by an increased growth of the epithelial cells at the lower part of the follicle. This growth becomes invaginated by a papilla of connective tissue, and the development proceeds as before; the new hair, helping to absorb and force the old one out, and, subsequently, taking its place in the original outer root sheath. The process is in many ways very comparable with the development of the permanent teeth, which, it will be remembered, cause the absorption of the lower part of the milk teeth and, ultimately, occupy their sockets.

The Nails.—A nail consists of two parts—a hard, horny layer, corresponding probably with the stratum lucidum of the skin—the stratum corneum being absent; and a softer, deeper layer—the Malpighian—continuous with the stratum Malpighii of the skin.

The dermis beneath the nail is divided into the *nail bed*, supporting the *body* of the nail, and the *matrix* beneath the *lunule*. Under the body it is disposed in the form of ridges running longitudinally, indications of which are obvious on the

surface of the nail to the naked eye. In the region of the matrix it is raised into papillæ with no definite arrangement. Down-growths from the Malpighian layer of the nail fill in the interval between the ridges and papillæ.

TERMINATIONS OF SENSORY NERVES IN THE SKIN.

Sensory nerves may terminate in (1,) general free endings; (2,) special tactile organs; (3,) neuro-epithelial cells. Only the first two of these divisions are found in the skin; the third includes the specialised epithelium of the organs of special sense.

1.—General free endings are found amongst the cells of the stratified epithelium of the skin and cornea. The nerve fibres lose all but the axis cylinder, which in its turn splits up into its constituent fibrillæ. The fibrils pass through the basement membrane and between the epithelial cells to terminate in free points or small knobs. Sometimes, as in the cornea, they form a network before doing so. They do not in the skin, or the walls of the hair follicles, penetrate beyond the Malpighian layer.

The *organ of Eimer* in the nose of the mole (*Fig. 143*) affords an instance of this mode of nerve termination. Here the epithelial layer is thickened by a downward prolongation at certain points, and these parts are pierced by a pencil of fibrils (*d*) from the dermis beneath, which penetrate to within a short distance of the stratum corneum. The fibrils show varicose thickenings, and terminate in small knobs (*f*). At the side of the epithelial thickenings other fibrils more widely separated from each other (*e*) pass into the epithelium in a similar manner. These fine nerve terminations are only revealed by the action of gold chloride. Another form of general free ending, one which is perhaps an approach towards a special tactile organ, is seen in the snout of the pig. Here the axis cylinder of the nerve fibre, or a division of it, divested of its sheath, after piercing the basement membrane, splits into fibrillæ, which terminate after branching in the lower part of the stratum Malpighii, in nucleated, somewhat semilunar discs, each of which supports an oval tactile cell.

In man similar *tactile cells*, somewhat pyriform in shape, have been described by Merkel in the deeper part of the stratum Malpighii. The cells are nucleated, and their lower pointed extremity is said to be joined by a division of the axis cylinder of a nerve fibre.

2.—**Special tactile organs.**—(*a*,) **Pacinian corpuscles** are found in the deeper part of the dermis, or more properly speaking, in the loose connective tissue between the cutis vera and the subjacent structures ; on the branches of the solar plexus in the mesentery ; about the joints, especially in the hand and foot ; and in other situations. They are comparatively large and elliptical in shape, being about $\frac{1}{2}$ in. in their long diameter, and $\frac{1}{4}$ in. in their short ; and are composed of a number of concentric laminae, surrounding a central cylindrical core, traversed by the termination of the axis cylinder of a nerve fibre. Each lamella is of the nature of ordinary connective tissue, and consists of delicate white and elastic fibres, with a large proportion of watery fluid between them. They are continuous for the most part with the perineurial sheath of the nerve fibre, and are separated from each other by lymph spaces lined with epithelioid cells.

The core consists of a homogeneous ground substance, containing granules or nuclei in its outer part. At the point at which the nerve fibre joins the pole of the corpuscle, the lamellae of its perineurial investment, and its grey sheath, expand suddenly to form the concentric lamellae of the Pacinian body, or, at least, those of its outer part. The innermost lamellae are more closely applied to each other than the outer, and are thinner, and they appear to be unconnected with the perineurium ; that is to say, there are more lamellae in the corpuscle than in the perineurium of the nerve, the inner being the additional ones, and these terminate in a free margin surrounding the penetrating nerve.

The nerve fibre retains its medullary sheath till the core is reached, *i.e.*, till it has passed through the series of envelopes, when it is continued as an axis cylinder alone to the other end of the corpuscle, where it frequently terminates in a thickened extremity. But the termination may be broken up into processes, or the fibre may bifurcate in its passage through the core.

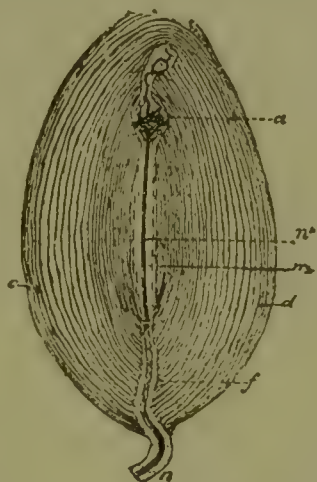


Fig. L.—Pacinian corpuscle from mesentery of cat : *n*, nerve fibre in sheath of Henle ; *n'*, its continuation through the core (*n*) as a naked axis cylinder ; *a*, termination of nerve ; *d*, lines of separation of tunics ; *c*, external lamellae ; *f*, canal through lamellae, traversed by nerve fibre (RANVIER).

FIG. 136.

S. SKIN (HUMAN), SHOWING SECTIONS OF SWEAT GLANDS AND DUCTS,
STAINED WITH HÆMATOXYLIN AND EOSIN $\times 300$.

- a.*—Sweat gland (secretory portion) cut longitudinally.
- b.*— " " " " " transversely.
- c.*—Secreting cells.
- d.*—Non-striped muscle fibres.
- e.*—Basement membrane.
- f.*—Irregular lumen of secreting portion.
- g.*—Duct.
- h.*—Fat cells.
- i.*—Connective tissue of cutis vera.

FIG. 137.

V.L.S. FÆTAL FINGER, STAINED WITH HÆMATOXYLIN AND PICRIC
ACID $\times 15$

- a.*—Nail, not yet free at the end.
- b.*—Root of nail.
- c.*—Terminal phalanx.
- d.*—Sweat gland.

Fig. 136.

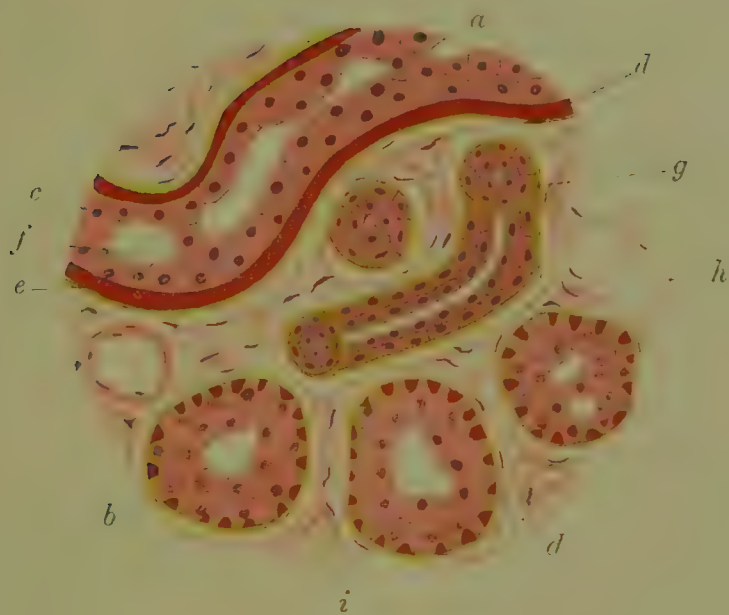


Fig. 137.



(b,) **Wagner's or touch corpuscles** are found especially in the papillæ of the skin of the palmar surface of the fingers. They are much smaller than the foregoing, being about $\frac{1}{300}$ in. long and $\frac{1}{800}$ in. broad. They are frequently somewhat pyriform in shape, the broad end being directed towards the surface. Their structure is still rather a matter of doubt, and two views may be regarded as being in most favour. According to the first the corpuscle is composed of a basis of connective tissue, forming a capsule from which project inwards more or less perfect transverse septa. As the nerve fibre (or there may be more than one) reaches the proximal end of the body it loses its grey and



Fig. M.—Wagner's corpuscle from skin of hand, stained with chloride of gold, and highly magnified: *n* nerve fibres; *a*, terminal ramifications of nerve fibre (RANVIER).

medullary sheaths, and immediately, or after first winding round the connective tissue basis, plunges into its substance, and gives off transversely running branches, which lie between the connective tissue septa described, and terminate in round or oval enlargements near to the periphery of the corpuscle. According to the second view, the corpuscle is composed of a series of superimposed cells, not unlike those of Grandry's corpuscles in the duck's tongue, enclosed in a connective tissue capsule, continuous with the perineurium of the nerve. The transverse markings upon the corpuscle, according to this view, result from the branches of the axis cylinder of the nerve fibre,

which run horizontally between two adjacent cells, and terminate in an enlargement, not so marked, but somewhat resembling the end plates in a Grandry's corpuscle.

(c,) **End bulbs** occur in the subepithelial tissue of the conjunctiva, of the lips and mouth, and the rectum. A special form is found beneath the epithelium of the glans penis and clitoris.

The end bulbs of the conjunctiva are spheroidal in shape, and consist of a core, formed of polygonal, or oval, nucleated cells, amongst which the axis cylinder (or there may be two) of the nerve fibre terminates; invested by a capsule, of which the outer fibrillated portion is continuous with the sheath of Henle of the nerve fibre, and the inner nucleated membrane with the sheath

of Schwann. As the nerve fibre reaches the bulb the medullary sheath ceases and the axis cylinder plunges into the capsule to end, after various convolutions, in a thickened blunted extremity. But the nerve fibre may divide before reaching the corpuscle, in which case the divisions are frequently twisted round each other as they enter it, and terminate in separate enlargements amongst the cells of the core.

(*d*.) **Grandry's corpuscles** are found beneath the epithelium of the mucous membrane of the mouth in some aquatic birds, such as the duck. They possess a fibrous capsule, continuous with the perineurium, or sheath of Henle, as it is called, when it surrounds a single fibre, lined by a nucleated membrane continuous

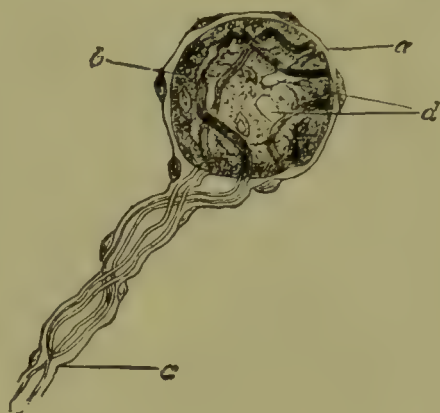


Fig. N.—End bulb from human conjunctiva, highly magnified: *a*, nucleated capsule; *b*, core; *c*, nerve; *d*, terminations of nerve (LONGWORTH).

with the sheath of Schwann, enclosing two, or sometimes three or more cells of a special character. They are nucleated, clear, or slightly granular, and disc-like in shape, and are placed with their flat surfaces apposed, but at the same time separated from each other by a biconvex tactile plate, which lies between them. As the nerve enters the corpuscle it loses its medullary sheath, and the

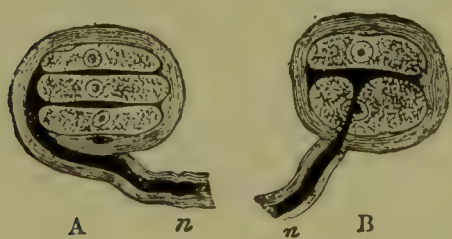


Fig. O.—Grandry's tactile corpuscles from tongue of duck, stained with gold chloride: A, composed of three cells with two intervening discs in which the axis cylinder of the nerve (*n*) ends; B, composed of two cells, with one intervening disc (RANVIER).

axis cylinder alone is continued along one side between the edges of the cells and the capsule. As it reaches the level of the interval between the discs it passes between them to end in the tactile plate. The latter seems to have no structural continuity with the cells it separates. Of course, where there are more than two cells there will be more than one intervening plate, and a corresponding number of axis cylinder terminations.

(*e*.) **Herbst's corpuscles** occur in the skin of birds, and are somewhat cylindrical in shape. The core consists of a number of nucleated cells, between which the axis cylinder passes as a

central stem. Outside the core are two fibrous layers forming a thick capsule, an outer arranged longitudinally, and an inner transversely, the latter being of a brownish colour.

(f,) **Corpuseles of Key and Retzius** are a transitional form between the Pacinian and Herbst's. They are found together with Grandry's in the mucous membrane of the mouth of the duck. Here the inner fibrous layer resembles greatly the concentric lamellæ of the Pacinian corpuscles.

Examine the following sections:—

(1,) *V. S. Human skin, palmar surface of the finger, stained with hæmatoxylin.* (Figs. 134 and 136.)

Place the specimen under the microscope so that the surface of the skin is away from you. Under the low power observe the following layers from above downwards (Fig. 134).

i.—The epidermis or epithelial portion of the skin consisting of—the stratum corneum (*a*), a thick homogeneous, lightly stained layer with depressions on its surface, indicative of the furrows which can be seen with the naked eye, and the corkscrew-like lumen of the sweat ducts (*d*), which open not in the furrows, but between them; the stratum lucidum (*b*), narrow and somewhat hyaline, and beneath it, often not well seen under this power, the stratum granulosum, narrow, dark and granular; the stratum Malpighii (*c*), a broad, deeply stained layer, dotted over with the nuclei of its cells. Observe the downward prolongations between the papillæ of the cutis vera; here and there one of these prolongations may be continued downwards as a sweat duct (*d*).

The layer of germinal cells, from the close apposition of its nuclei, gives a deeply stained lower edging to the stratum Malpighii.

ii.—The cutis vera or connective tissue portion of the skin. Notice that it is much less deeply stained than the epithelium above it, and that its surface is raised into papillæ, varying very much in size and shape. Even under the low power in some of these a Wagner's corpuscle may be seen, as a somewhat elongated, more deeply stained body. Observe that the connective tissue becomes more open and coarser in the deeper part of the dermis, where sections of blood-vessels, nerves, and lymphatics are easily made out. The Pacinian corpuscles (*g*) are very easily seen with the low power. They are situated rather deeply, and appear as

large round or oval bodies, often occurring in groups of two or three together. Note the concentric arrangement of the laminae enclosing a central core in which the nerve ends. The nerve fibre will not, of course, be seen entering the corpuscle unless the section has passed in the required plane, and in any case it is not easily seen unless stained with gold chloride. In this deeper part of the true skin, observe also the lobules of fat cells (*f*). The cells appear merely as outlines, unless osmic acid is used to stain them, when they are brown or black.

Finally, note the sweat glands (*e*), the coiled portion of which lies in the deeper part of the dermis. They form groups of variously cut portions of tubules, some of which are secretory, while others are ductular. From one of these groups a duct may be seen emerging and passing upwards to join one of the prolongations of the Malpighian stratum of the epidermis. The sweat glands are much more deeply stained than the connective tissue amongst which they lie.

Under the high power examine first the epithelial layers (*A*). (These are shown in *Fig. 135*, which is, however, taken from the skin of another part.)

Note the corneal layer (*a*) composed of superimposed, flattened squames. The outlines of the individual cells can sometimes, but not always, be made out. Here and there a flattened nucleus in section may be seen. The cells have been converted to keratin, and are virtually dead. They serve merely a mechanical protective purpose, and are constantly being removed from the surface by attrition. Here and there flakes of loosened cells may be seen in section. Observe the stratum lucidum (*b*), thin and homogeneous; the outlines of the cells forming it, and their nuclei, are very rarely revealed. Beneath the stratum lucidum look for the stratum granulosum (*c*). It is usually readily recognisable from its dark granular appearance. Note the diamond or lozenge shaped cells forming it, each of which shows a nucleus deeply stained, surrounded by well marked granules of *elcidin*, staining deeply with carmine, in the perinuclear protoplasm. Finally, examine the stratum Malpighii (*d*). Note the several layers of superimposed epithelial cells, the size and shape of which may be observed to vary according to their depth. The lowest layer of all, immediately covering the connective tissue papillae, is readily distinguished by the smallness of the cells and the comparative largeness

of the nuclei. The oval, deeply stained nuclei form a very distinct layer, set endwise as they are, in close proximity to each other, upon the basement membrane. This is the germinal layer of cells, from which those above are being constantly augmented. The middle, principal part of the stratum Malpighii, composed of large polygonal cells with spherical nuclei, should next be examined. To see the intercellular bridges or prickles connecting them with each other, draw the tube of the microscope out to its fullest extent, when the spaces between the ridges appear as a row of dots outlining the cells. With a power of 350 or 400 the student will not, as a rule, make out much more than this. In the upper part of the stratum Malpighii the cells approach those of the stratum granulosum in shape, being less polygonal and more lozenge shaped than those just described. They still, however, show the characteristic dotted outline of prickle cells.

Examine next the cutis vera (B), fine in the region of the papillæ, coarse and more areolar in texture in the deeper part. Deeply stained nuclei of connective tissue cells of various shapes are everywhere to be seen amongst the bundles of white fibrous tissue. Note the contents of the papillæ. Most of them are vascular, indicated by the deeply stained nuclei of the walls of the capillary vessels. In some a Wagner's corpuscle (*e*) may be seen with a nerve fibre (*f*) entering its lower extremity. The corpuscle may now be seen to owe its deeply stained appearance mainly to the presence of numerous nuclei placed transversely. Note also the fibrillation of the corpuscle, also transverse, due to the direction taken by the septa of connective tissue, and to a certain extent to the direction taken by the nerve fibre after its entrance. Those papillæ containing Wagner's corpuscles have no capillary network.

Find one of the Pacinian bodies in the deeper part of the dermis (*Fig. L*, p. 419). Note the very definite concentric laminæ with the nuclei of the epithelial plates between them. Observe the central part of the corpuscle. If the core has been cut transversely it will appear small and circular; if longitudinally, it will be seen extending through the middle half of the corpuscle. In the latter case the nerve fibre may, or may not, be seen entering one end of the corpuscle and penetrating to the core.

Examine the sweat glands and ducts (*Fig. 136*). Select one of the groups in the deeper part of the dermis. The glandular

FIG. 138.

T.S. HUMAN NAIL, STAINED WITH HÆMATOXYLIN $\times 100$.

- a.*—Horny layer.
- b.*—Malpighian layer.
- c.*—Cutis vera.

FIG. 139.

V.S. LIP OF KITTEN, SHOWING TACTILE HAIR, STAINED WITH HÆMATOXYLIN $\times 40$.

- a.*—Hair.
- b.*—Outer dermic covering, enclosing sinus (*k*).
- c.*—Outer root sheath (continuous with stratum Malpighii of epidermis).
- d.*—Inner root sheath $\left\{ \begin{array}{l} e.—\text{Henle's layer.} \\ f.—\text{Huxley's } ,, \end{array} \right.$
- g.*—Dermic papilla.
- h.*—Nerve piercing dermic covering.
- i.*—Sebaceous glands (rudimentary).
- k.*—Sinus between layers of dermic covering.

Fig. 138.

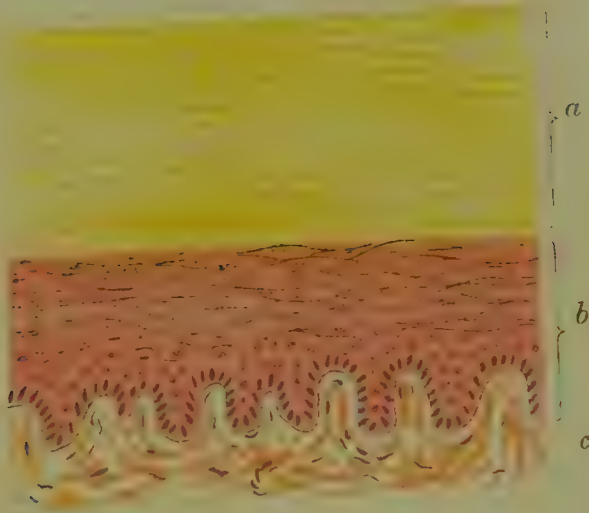
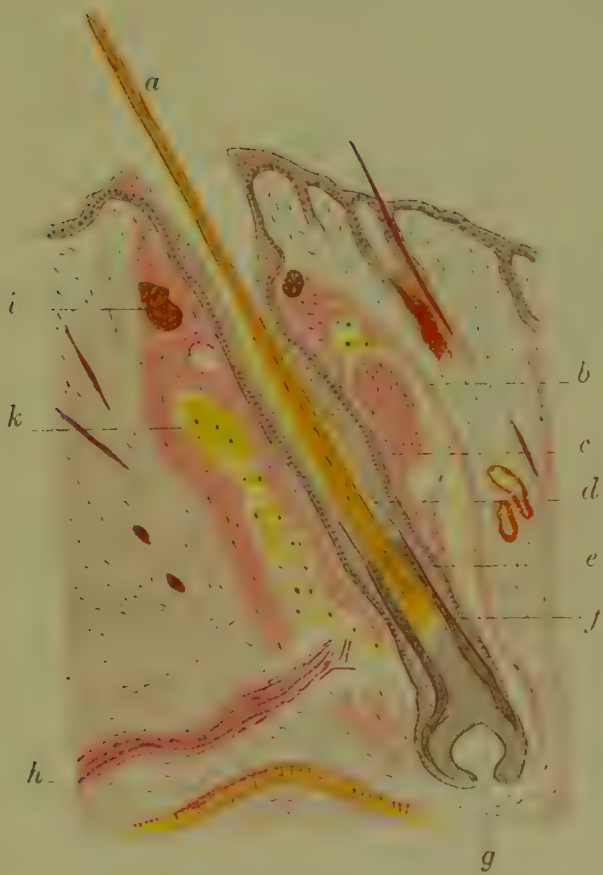


Fig. 139.



or secreting part (*a, b*) of the tube may be at once distinguished from the coiled part of the duct (*g*) by the fact that it is larger, the epithelium lining it does not stain so deeply, and non-striated muscle fibres are found within the basement membrane. Find a transverse section of the glandular part (*b*), and observe the irregular lumen, surrounded sometimes by more than one layer of nucleated, somewhat irregular glandular cells. The whole is enclosed in a well marked basement membrane, within which small somewhat triangular points (*d*) are seen at regular intervals. These are transverse sections of the longitudinally or spirally running fibres, and are very characteristic of the secreting portion of the tube. They are stained a pink mauve colour with hæmatoxylin, which, on the addition of eosin, becomes a distinct red.

Examine also a longitudinal section of the glandular portion (*a*), in which the muscular layer (*d*) is continuous and not interrupted.

If a transverse section of one of the ducts be examined it will be seen to be smaller than that of the secreting portion; the lumen is narrower and regular; and no muscular fibres are present between the cells and the basement membrane. The ducts stain more deeply with the hæmatoxylin than do the gland tubules.

Trace one of the ducts seen passing vertically from one of these groups to the epidermis. Notice that it continues to be lined by two layers of somewhat cubical, nucleated cells, and that at the point where it joins one of the downward prolongations of the stratum Malpighii, these become continuous with the cells of that layer. Trace the lumen of the duct through the epidermis, where it forms a channel amongst the epithelial cells, noting the remarkable corkscrew character it assumes in traversing the stratum corneum. The basement membrane of the duct fades off into the surface condensation of the cutis vera surrounding the Malpighian prolongation.

(2.) *V. S. Skin of palmar surface of finger, hardened in osmic acid, and stained with borax-carmin.* B.

With the high power examine the stratum Malpighii for prickles. Look also for Wagner's corpuscles in the papillæ of the dermis.

(3.) *V. S. Skin of negro, stained with picro-carmin.* F.

Note the granules of melanin pigment in the cells of the

deeper layer of the stratum Malpighii. It is to the presence of these granules that the dark colour of the skin is due.

(4.) *V. S. Skin of fœtus, stained with osmic acid. F. (Fig. 24, B.,*

Note under the low power the groups of fat cells in the lower part of the dermis. The fat is stained black with the osmic acid. Even under this power it can be seen that the cells vary very much in size. Select a small group and put on the high power. Note the fat cells in various stages of development. They commence as connective tissue corpuscles, in which a small droplet of oil appears. This is succeeded by others. In a little time the droplets coalesce to form one or two larger ones, and these finally unite to form the single globule of the fully developed fat cell. In such a cell all the protoplasm has become converted to fat, with the exception, perhaps, of a small amount around the nucleus. As the fat accumulates the remaining protoplasm and the nucleus are pushed to one side of the cell, and constitute the signet of the ring, the envelope constituting the hoop. In any group, cells showing the various stages may easily be found.

Look, too, for developing capillary blood-vessels. Many of the connective tissue cells, instead of developing oil in their interior, may be seen to be forming a branching network, which is becoming vacuolated here and there to form the lumen of the future vessel. (*See "Development of blood corpuscles," page 81.*)

(5.) *V. S. Lip of kitten, stained with hæmatoxylin. B. (Figs. 139 and 140.)*

The large hair follicles of the lip of a kitten are not precisely similar to those of the human scalp. They are, of course, larger; they are surrounded with a blood sinus, and the sebaceous glands are rudimentary; but, partly perhaps on account of their large size, the structure of the different layers of the hair follicle can be more readily made out in them. Place the section with the skin surface away from you when seen under the microscope, and under the low power (*Fig. 139*) study the following points: The thin layer of epidermis on the surface, much thinner than that covering the palmar surface in the human subject; the cutis vera beneath, much more lightly stained, containing several large hair follicles, cut in longitudinal section for the most part, with numerous smaller ones between them. The figure shows one of the large hair follicles in complete longitudinal section. Select such a follicle, and note its different parts as far as they can be

made out under this power. Observe the blood sinus (*k*), limited by a fibrous investment (*b*), surrounding the sides of each follicle; the outer epithelial covering of the follicle (*c*) continuous with the stratum Malpighii of the epidermis; within this the inner root sheath (*d*), homogeneous and almost unstained in its upper part, deeply stained in the lower; and within this the hair itself (*a*). Trace the hair, which usually shows some pigmentation in the lower part of the follicle, downwards till it joins the invaginated bulb of epithelium from which it springs. Notice that the inner and outer root sheaths, when traced downwards also end in this bulb. Observe the papillæ of connective tissue (*g*), part of the dermis, filling the invaginated end of the bulb. The papilla is readily distinguished from the epithelium of the bulb by reason of the latter being very much more deeply stained.

Finally, observe the rudimentary sebaceous glands (*i*) opening into the mouth of the follicle, their saccular portion being so small that it merely occupies a space in the thickened dermic covering; the nerve (*h*) piercing the dermic covering in its lower part and traversing the sinus; the oblique position of the whole of the follicle. Under the high power (*Fig. 140*) examine first the layers of the hair follicle from without inwards. First note the dermic or fibrous coverings, here separated into two distinct layers (of which only the inner (*a*) is shown in the figure) of connective tissue by the blood sinus. Examine the contents of the sinus, observing the trabeculæ of the connective tissue network traversing it, covered with epithelial plates, the nuclei of which are usually very distinct. The meshes of the network are filled with blood corpuscles. Next, identify the glassy hyaline membrane (*c*), and trace it down over the surface of the papilla (*h*). Within the hyaline membrane the outer root sheath (*d*) is readily recognisable, composed of two or three layers of cells, of which those next the hyaline membrane are similar to the germinal cells of the stratum Malpighii of the skin. The inner root sheath may next be examined, and its differentiation into two layers in its lower part, and their structure, made out. First study the inner root sheath immediately above the bulb. Notice on the inner side of the outer root sheath a layer, lightly stained, narrow, refractile (*e*), rather like the hyaline membrane, but a little broader. This is the outer division of the inner root sheath, the layer of Henle. It consists of one or two layers of flattened

FIG. 140.

L.S. HAIR FOLLICLE OF LIP OF KITTEN, STAINED WITH HÆMATOXY-
LIN $\times 300$.

- a, b.*—Inner dermic covering.
- c.*—Hyaline membrane.
- d.*—Outer root sheath.
- e.*—Henle's layer. }
- f.*—Huxley's " }
- } Inner root sheath.
- g.*—Cuticle of hair.
- h.*—Papilla of cutis vera.
- i.*—Hair.

FIG. 141.

T.S. HAIR FOLLICLES AT DIFFERENT LEVELS, STAINED WITH HÆMA-
TOXYLIN $\times 300$.

A.—Below opening of sebaceous glands.

- a.*—Fibrous part of dermic covering.
- b.*—Hyaline membrane.
- c.*—Outer root sheath.
- d.*—Inner root sheath.
- f.*—Hair.

B.—Above apex of papilla.

- a.*—Fibrous dermic covering.
- b.*—Hyaline membrane.
- c.*—Outer root sheath.
- d.*—Henle's layer.
- f.*—Huxley's layer and cuticle.
- g.*—Commencement of hair, showing pigmentation.

Fig. 140.

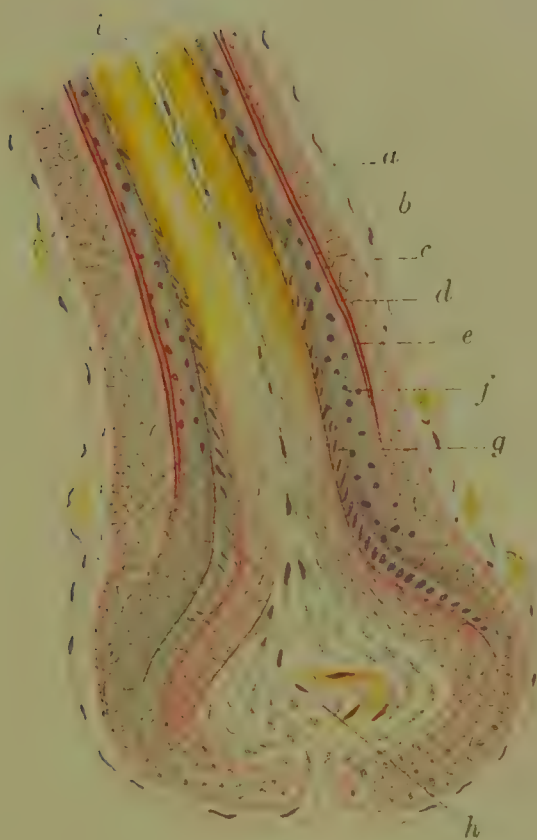
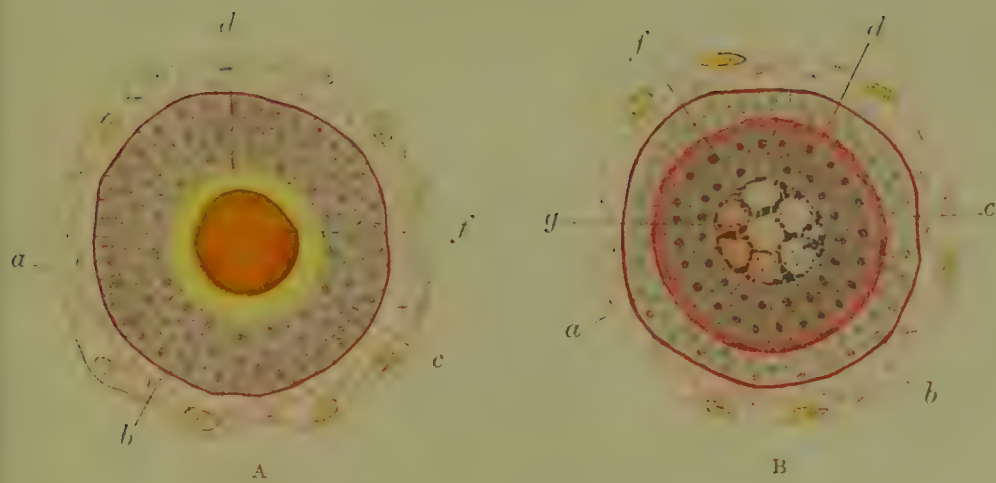


Fig. 141.



nucleated cells, the outlines of which cannot usually be made out. Flattened nuclei (in section) may sometimes be seen here and there. Within this look for Huxley's layer (*f*). This is more easily recognised, as it stains deeply with the hæmatoxylin.

Huxley's layer will be seen to consist of two or more layers of somewhat cubical or polygonal cells with very distinct nuclei. The nuclei stained deep blue are very frequently quadrangular in outline, possibly as the result of the action of the reagent. Trace these two layers, Henle's and Huxley's, upwards, and note that about the middle of the length of the follicle they fuse together, become homogeneous, and stain very lightly or not at all. Next, look for the commencement of the cuticle (*g*) of the hair itself. This is readily seen in the bend formed by the junction of the hair with its expanded base in the bulb. Note the tall columnar shaped cells placed slantingwise on the shoulder of the expansion, overlapping each other from below upwards. Follow them upwards on to the surface of the hair, where they appear to fade away into a thin line; and downwards into the bulb, where they become more cubical, and are lost among the other layers. Notice that the cells are imbricated upwards on the hair, but as they sink into the bulb they assume a direction vertical to its surface. They are nucleated in the lower part of the follicle; when they form the cuticle of the hair they are non-nucleated periplasts only. Next observe the hair itself. Above the bulb its cells have become elongated and fibrous, and are non-nucleated; in the bulb itself they are polygonal and nucleated, with the exception of the lowest layer of all immediately covering the papilla. These cells are cubical or columnar, and are continuous with the basal cells of the outer root sheath, *i.e.*, with those which in their turn are continuous with the germinal layer of the stratum Malpighii of the skin.

Thus the cellular layers of the hair follicle, and the hair itself when traced downwards, lose their identity in the bulb, with the exception of the basal columnar cells of the outer root sheath, which are continued over the papilla. (In the drawing the continuity of these cells is, however, interrupted by the prolongation of the apex of the papilla, as a core to the hair—a not unusual variation.)

Examine the fibrous papilla (*h*), noting the number of small deeply stained nuclei of the connective tissue cells, and sections of capillary blood-vessels.

(6,) *V. S. Scalp of child, stained with hæmatoxylin. B. (Fig. 142.)*

This specimen may be examined for the structure of the hair follicle in the same way as the previous one. It shows particularly well the position and structure of the sebaceous glands, and the erector pili muscle.

Under the low power note first that the hair follicles are not enclosed, as in the case of the large tactile hairs of the cat, in a blood sinus. Observe the surface epithelium (*b*) as it dips down to form the mouth of the hair follicle, and note its continuity with the outer root sheath (*e*). Find the sebaceous glands (*d*) opening into the neck of the follicle, one at each side. They surround the follicle at this point in reality, but in section they appear to be only on the two sides. Under this power observe, too, the position of the erector pili muscle (*c*) on that side of the follicle which forms an obtuse angle with the surface of the skin. It arises from the superficial part of the cutis vera beneath the stratum Malpighii, and passes down to be inserted into the hyaline membrane below the level of the sebaceous gland of that side, which it thus encloses in the angle.

Put on the high power and study the structure of the sebaceous glands. Note that they are solid sacs of polygonal cells, the central ones of which undergo fatty degeneration to supply the sebaceous secretion, their place being supplied by cells developed from the peripheral layers. Note the very distinct intra-cellular network of these cells. The gland alveoli are largest on the side of the erector pili muscle.

Study the structure of the erector pili muscle, noting the rod-shaped nuclei of the muscle cells.

Observe the homogeneous, translucent looking upper portion (*f*) of the inner root sheath, and that it cannot be traced higher than the opening of the sebaceous glands.

(7,) *T. S. Scalp of child, stained with hæmatoxylin. B. (Fig. 141.)*

In this specimen very instructive transverse sections of a hair follicle, taken at different levels, are to be seen. In the figure (A) is taken at a level somewhat below the opening of the sebaceous glands. Observe especially the inner root sheath, consisting of the layers of Huxley and Henle, fused and homogeneous (*d*). (B) is taken at a level just above the bulb. Note the hair in the centre still cellular (*i.e.*, the cells not yet sufficiently elongated to form fibres), and often at this level pigmented, the pigment

granules being between the cells ; outside it Huxley's layer and the cuticle of the hair (apparently forming one layer), composed of somewhat cubical or polygonal cells (*f*) ; outside it Henle's layer (*d*), in which the outlines of the flattened cells, and the nuclei of some of them may be seen. In the section at any level the outer root sheath (*c*), the hyaline membrane (*b*), and dermic covering (*a*), can readily be distinguished.

(8.) *V. L. S. Fœtal finger, stained with picric acid and hæmatoxylin. B. (Fig. 137.)*

It is convenient to study the nail in a fœtal finger, because it is so much more easily cut than in the adult, and also because the method of development is indicated.

Ascertain by looking at the specimen with the naked eye on which side the nail lies, and place it under the microscope so that on looking through the tube it may be away from you.

Under the low power (*Fig. 137*) recognise first the different parts of the section. Observe the epidermis enveloping the finger, except where the nail occurs ; it is everywhere thin except just in front of the anterior margin of the nail, and in this region the stratum corneum is considerably thickened. Here, too, the stratum lucidum and stratum granulosum are most distinctly seen. Notice next the presence of numerous sweat glands and ducts (*d*) lying in the dermis of the palmar surface. Observe especially the sweat ducts which are much more in evidence here than in adult skin. Observe the nail as a narrow homogeneous band (*a*), stained brightly with the picric acid, resting upon the Malpighian layer stained with the hæmatoxylin. Observe the root of the nail (*b*), placed posteriorly and covered by a fold of epidermis, as apposed to the body, the more anterior and larger portion, the surface of which is exposed. Trace the body to its anterior edge, and note that it is not yet free.

Note the longitudinal sections of the terminal and other phalanges occupying the central part of the specimen. They usually afford a good illustration of L.S. developing bone, the blood sinuses in the marrow being often particularly well seen.

Under the high power examine the stratum Malpighii of the nail. Note that its lower border is a straight one in the greater part of its extent, but that at the root of the nail the epithelium shows downward prolongations between corresponding papillæ of the connective tissue or dermis beneath. At the anterior edge

of the nail, where the stratum Malpighii joins the same layer of the skin, the same downward prolongations are seen also.

Observe the connective tissue beneath the nail and the epidermis of the finger generally. It is raised into papillæ everywhere, except beneath the body of the nail.

(9.) *V. T. S. Adult nail, stained with picric-acid and hæmatoxylin. B. (Fig. 138.)*

The section should be taken a little anterior to the lunule. Under the low power observe the horny layer (*a*), a broad, homogeneous band, stained brightly with the picric acid; beneath it a narrow layer, the stratum Malpighii (*b*), stained with the hæmatoxylin, resting upon the dermis or connective tissue basis of the skin (*c*). Observe that the dermis appears to be raised into small papillæ of a fairly uniform size and shape. All the papillæ are simple. These are not, however, in reality papillæ; they are transverse sections of the ridges of connective tissue running longitudinally beneath the stratum Malpighii of the nail.

Under the high power study the structure of the parts more minutely. The horny part of the nail is virtually homogeneous, and does not show the outlines of the flattened cells of which it is formed. It represents either the stratum corneum or lucidum of the epidermis, or it may be both. The stratum granulosum is absent.

Note the germinal layer of the stratum Malpighii. The cells above show no prickles, in this respect differing from those of the stratum Malpighii of the skin. They are also smaller in size.

(10.) *V. S. Epidermis of snout of pig, stained with gold chloride.*

Under the high power observe a nerve fibre stained purple with the reagent, devoid of its medullary sheath, splitting into fibrils as it enters the stratum Malpighii between the cells. The fibrils may be seen terminating in semilunar, nucleated discs, each of which supports a tactile cell. The discs in section are semilunar, in reality saucer-shaped, with the concavity directed upwards. They are found especially in the deeper part of the stratum Malpighii. The nerve fibres branch repeatedly.

(11.) *V. S. Skin of palmar surface of finger, stained with gold chloride.*

Note the purple nerve fibrils penetrating the stratum Malpighii between the cells, branching and terminating in free points. Small varicosities are seen upon them here and there.

(12,) *V. S. Skin of nose of mole, stained with gold chloride. B. (Fig. 143.)*

Under the low power note the prolongations of the epithelium downwards into the dermis, constituting the *organs of Eimer*. Select one of these and put on the high power. Observe the nerve fibre in the dermis reaching the epithelium, and the leash of nerve fibrils (*d*) passing vertically through the stratum Malpighii towards the stratum corneum, which it does not, however, reach. Observe the varicosity of the fibrils, and that they frequently end in a small head or thickening, apparently unconnected with the epithelial elements. At the side of the organ of Eimer note that some of the nerve fibrils (*e*) penetrate the epithelium apart from the central leash, and pursue a separate course.

(13,) *V. S. Fœtal skin from palmar surface of finger (for Pacinian corpuscles), stained with hæmatoxylin. B. (Fig. 134)*

Under the low power observe one or more of the concentrically marked Pacinian corpuscles in the subcutaneous tissue. Put on the high power and examine. The appearance presented by a corpuscle will depend upon how it has been cut. If longitudinally and through the centre, the length of the core may be seen; if transversely, the core cut transversely also, will occupy a smaller area; if obliquely, the appearance will be an intermediate one; or the core may not be seen at all if the centre of the corpuscle has been missed. Study the concentric laminæ, noting the presence of nuclei stained pink between them. These are the nuclei of the epithelium covering either side of each lamina, *i.e.*, the epithelium lining the space between adjacent ones. The structure of the core is not usually well made out in this specimen, but the axis cylinder should be looked for in a corpuscle cut longitudinally (*Fig. L, p. 419*).

A section, vertical or transverse, of the paw of a kitten, stained with picro-carminé or hæmatoxylin, shows usually a considerable number of Pacinian corpuscles about the joints. The paw requires previous softening.

(14,) *V. S. Conjunctiva (human, for end bulbs), stained with picro-carminé or hæmatoxylin. F. or B.*

Use the human conjunctiva in preference to that of a calf, as the end bulbs are more typical. It is not easy to see the terminations of the nerves in these organs or in the Pacinian bodies, unless the specimen is stained with gold chloride; at the same

FIG. 142.

V.S. HUMAN SCALP, STAINED WITH HÆMATOXYLIN \times 100.

- a.*—Hair.
- b.*—Epidermis.
- c.*—Erector pili muscle.
- d.*—Sebaceous gland.
- e.*—Outer root sheath.
- f.*—Inner root sheath.

FIG. 143.

V.S. EPIDERMIS OF NOSE OF MOLE, TO SHOW EIMER'S ORGAN,
STAINED WITH GOLD CHLORIDE \times 300.

- a.*—Down-growth of epithelium.
- d.*—Leash of nerve fibrils.
- f.*—Nerve fibrils terminating in knobs.
- e.*—Nerve fibrils entering sides of down-growth.

Fig. 142.

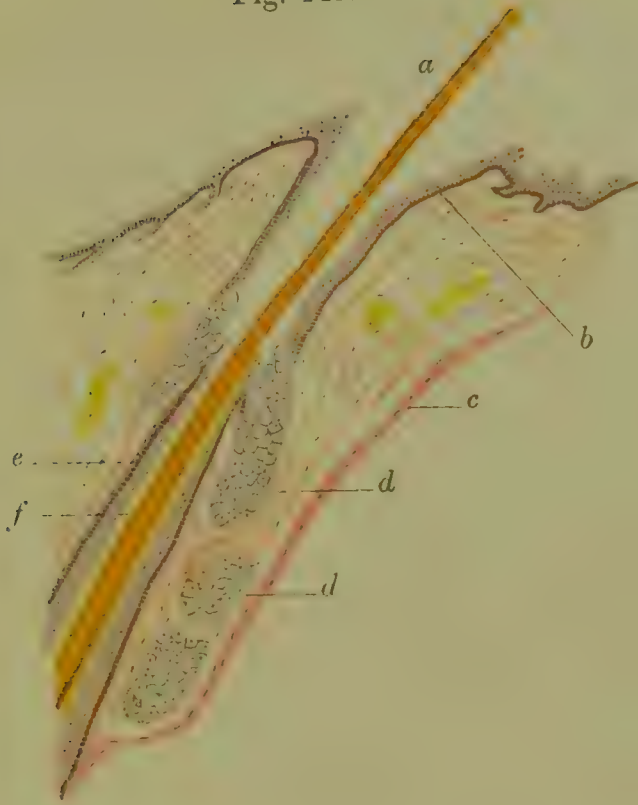


Fig. 143.



time, a very fair general idea of the structure may be obtained from a section stained with ordinary reagents, especially if it be a thin one. Find one of the bulbs in the connective tissue beneath the epithelium. Note its nucleated capsule, and the somewhat indefinite appearance of the core. If possible, trace a nerve fibre, or the two divisions of one, to a bulb.

(15,) *V. S. Tongue of duck (for Grandry's corpuscles), stained with picro-carmin or hæmatoxylin. F. or B.*

Arrange the specimen on the stage so that on looking through the tube the dorsum of the tongue may be away from you. To either side of the dorsum look for Grandry's corpuscles in the connective tissue beneath the epithelium. Usually, one at least can be seen on either side of the tongue; and often in the same neighbourhood a specimen of Key and Retzius' corpuscles. Put on the high power, and identify in Grandry's corpuscle the capsule of connective tissue, and especially, if possible, the two or three disc-like cells inside it. Again, the appearance of the corpuscle will depend upon how it has been cut. The two or three cells may be seen in section, or only one of them may have been cut. If the cells have been divided vertically to their chief plane, look for a nucleus in each, and note the clearness of the cell substance. The line between two cells indicates the position of the end plate in which the nerve fibre terminates. The latter cannot in this specimen be traced into the corpuscle, but may possibly be seen reaching it through the connective tissue of the dermis. Examine a corpuscle of Key and Retzius, if this be present. It is readily recognised from the concentric arrangement of its laminæ. Note how much it resembles a Pacinian body, except that it is much smaller. Observe especially the core, and note that it is rather different from that of a Pacinian body. If cut in longitudinal section, it shows a row of nuclei on each side of the central axis cylinder.

APPENDIX TO CHAPTER XIV.

METHODS OF PREPARATION.

1. Y.S. Skin from palmar surface of finger.—Harden in Müller and spirit ; cut in gum, stain in picro-carmine or hæmatoxylin, and mount in Farrant or balsam ; or stain in bulk in borax-carmine, and cut in paraffin.

2. Y.S. Skin for prickle cells.—Harden small pieces from palmar surface of fingers in osmic acid for 24 hours, and complete in spirit ; cut thin sections in gum or paraffin, and mount in Farrant's solution without further staining ; or stain in bulk in borax-carmine, and cut in paraffin.

3. Y.S. Scalp of child for hair follicles.—Harden in Müller and spirit ; cut in gum, stain in hæmatoxylin, and mount in balsam ; or stain in bulk in borax-carmine, and cut in paraffin. Care requires to be taken to cut the sections in the axis of the follicles.

4. Y.S. Lip of kitten for tactile hairs.—Take the whole thickness of the upper lip, with the "whiskers" projecting from it. Cut these off close to the skin with a pair of scissors. Treat in the same way as 3.

5. T.S. Hair follicles.—Take pieces of 3 and 4, and cut transversely to axis of hairs. Mount sections taken at different levels.

6. T.S. Nail of foetus.—Harden in Müller and spirit, and decalcify in chromic and nitric fluid, the finger of a foetus, in which the nail is not yet free at the end. Cut in gum, stain in picric acid and hæmatoxylin, and mount in balsam. The advantage of the picric acid, is that in this way the corneous substance of the nail is brought into relief. The alcohol used for dehydrating should contain picric acid. This specimen frequently shows very beautiful longitudinal sections of developing phalanges.

7. Free nerve terminations in Malpighian layer of epidermis.—Place small pieces of skin from the palmar surface of finger of child in boiled gold chloride and formic acid for an hour, and expose to light till reduction takes place, in acidulated water. Harden in alcohol, cut in paraffin, and mount in balsam. (See methods of staining with gold chloride, page 47).

8. Nose of mole ; snout of pig ; skin of finger for Pacinian and touch corpuscles ; conjunctiva ; mucosa of tongue of duck ; may all be tried (1,) by the gold chloride method, being hardened in spirit after staining, and cut in paraffin. The student is recommended to try each gold chloride method successively till he finds one which works well in his hands, and to adhere to that method. (2,) Harden in Müller and spirit, cut in gum, and stain in picro-carmine or hæmatoxylin ; or preferably stain in bulk in borax-carmine and cut in paraffin. The nerve fibres are not, of course, specially revealed by this method, so that it is not of much service for demonstrating free nerve endings ; but Wagner's touch corpuscles, Pacinian corpuscles, end bulbs, and Grandry's corpuscles may all be fairly well shown by it.

CHAPTER XV.

THE REPRODUCTIVE SYSTEM.

THE MALE ORGANS.

The Testis.—The testis, like the kidney, is a compound tubular gland, but the length of the ducts is a more marked characteristic than that of the tubules. It is invested with a serous sac, similar to the peritoneal, the visceral layer of which is termed the *tunica vaginalis*. Beneath the tunica vaginalis lies the *tunica albuginea*, a tough, fibrous investment continuous with the general connective tissue framework of the organ. When traced backwards (*Fig. 144*), this coat passes into the gland to form the *corpus Highmorianum*, or *mediastinum testis*, from which fibrous septa are continued towards the inner surface of the capsule. The result of this arrangement is that the organ is split up into some hundred and twenty compartments or loculi, in which the secreting tubules lie. The figure shows the arrangement diagrammatically: (*e*) indicating one of the radiating septa, and (*d*) the tubules lying in one of the compartments. Each of these contains some two to eight convoluted tubules, which begin blindly beneath the capsule, and after running a tortuous course towards the apex of the compartment, narrow to form the *tubuli recti*. The latter pass into the mediastinum and anastomose freely with each other to form the *rete testis* (*g*, see also *d*, *Fig. 145*). From the rete testis the secretion of the tubules, or *tubuli seminiferi*, as they are sometimes called, is conveyed by ten to fifteen wider *vasa efferentia*, the terminal parts of which become convoluted to form the *coni vasculosi*. These unite to form a single tube, the *epididymis*, which is of great length and elaborately coiled upon itself to form an eminence at the back of the testis. The continuation of the epididymis to the *vesiculæ seminales* is termed the *vas deferens*.

The tubules are enclosed in a well-defined basement membrane, which supports two, three, or more layers of epithelial cells, the nature of which will be described immediately. The lumen of the tube is occupied by the secretion of the testis, the semen, containing the spermatozoa. A spermatozoön, the male element required for the impregnation of the ovum, has the following characters: it possesses a brightly refractile *head* of a somewhat oval shape, and a long cilium, or *tail*. The head is somewhat broader at its base, where it joins the tail, than at its anterior extremity. The part immediately succeeding the head is somewhat thicker than the rest of the tail, and is termed the *neck* or *body*. The head represents the nucleus of a cell, or part of the nucleus; the tail, probably part of the cell protoplasm. The lumen of the tubule is occupied by the spermatozoa, which are arranged in fan-shaped collections, or in a continuous row. They are disposed in a radial manner, the heads being imbedded among the epithelial cells lining a tubule, and the tails projecting into the centre. The vasa recta are lined by a single layer of cubical cells, which in turn become flattened when the rete is reached. The basement membrane, which is well marked, and even frequently in more than one layer, where it surrounds the tubules, in the rete has lost its identity, and is scarcely distinguishable from the surrounding connective tissue. The vasa efferentia proceeding from the rete to the epididymis have well defined walls of connective tissue, circular muscular fibres being also present, and are lined with ciliated columnar cells. The structure of the tube where it forms the epididymis is still more distinct, the epithelial cells being particularly well formed and their cilia of unusual length. In the vas deferens the muscular development has reached its maximum, there being a thick, inner, circular coat, and a thinner, outer, longitudinal one. The cells of the epithelial lining, however, are no longer in a single layer, and they do not possess cilia.

Spermatogenesis.—The exact history of the development of the spermatozoa from the cells lining the seminiferous tubules has long been a matter of considerable difference of opinion, nor can we even now say that the information on the subject is in any way complete and final. The following account explains several of the appearances to be seen in the tubules in a fairly simple manner, but it must be borne in mind that the theory is only one out of several that have been advanced by different observers.

The changes in the cells of the tubules are illustrated in Fig. 146, and Figs. *P* and *Q*, of which the first is after nature, the second diagrammatic, and the third semi-diagrammatic.

In a tubule in which the process of spermatogenesis is in a full state of activity, there are four layers of cells to be considered if the spermatozoa themselves rank as one.

The *outer layer* is represented (Fig. *P*, *a*) by a single row of cubical nucleated cells placed immediately within the basement membrane. These cells are termed *spermatogonia*, and are the only ones found in the early foetal testis.

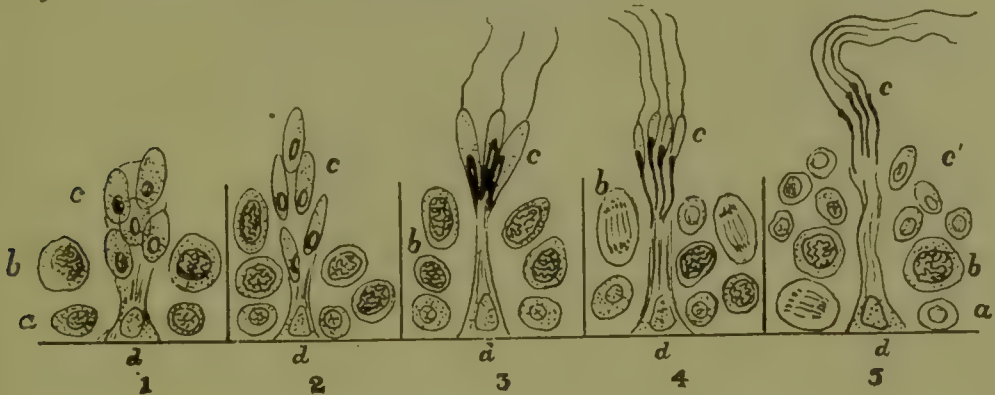


Fig. *P*.—Diagrammatic representation of spermatogenesis in the rat : (*a*,) layer of spermatogonia ; (*b*,) layer of spermatogens ; (*c*,) spermatoblasts in course of development to spermatozoa. 1, 2, 3, 4, 5, show progressive stages of this development ; in 5 is shown the second crop of spermatoblasts (*c'*) ready to take the place of the spermatozoa (*c*), developed from the first, as soon as they are discharged.

The *second* or *intermediate layer* (*b*) consists of a number of spermatogens derived by division from the first. They are large, clear cells, and usually show a well marked "mitotic" nucleus, often in the wreath stage. These cells may be in a single layer, or they may be two or three deep. But the original lining of spermatogonia does more than give origin to these spermatogens. Some of the cells appear to be set apart for another purpose, viz., that of elongating and extending radially towards the centre of the tubules, to form *sustentacular* columns. These are placed in the circle of the tubule much in the same way as the spokes in a wheel.

We shall return to these sustentacular cells (*d*) immediately ; it need only further be noted now that they necessarily break up the layer of spermatogens (*b*) which thus lie in groups between them.

The third layer of cells is composed of *spermatoblasts* (1 *c*), which are frequently more numerous than the last, from which

FIG. 144.

S. TESTIS OF KITTEN (DIAGRAMMATIC) $\times 20$.

- a.*—Tunica albuginea.
- b.*—Serous cavity.
- c.*—Tunica vaginalis.
- d.*—Lobules of tubuli seminiferi.
- e.*—Fibrous septa between the lobules.
- f.*—Tubuli recti leading into,
- g.*—Rete testis.
- h.*—Fibrous partition between testis and epididymis.
- i.*—Section of epididymis.

FIG. 145.

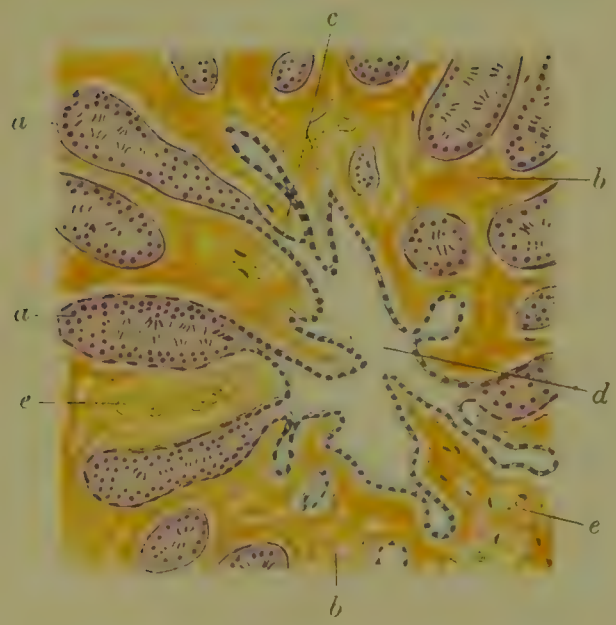
S. TESTIS OF RABBIT, THROUGH RETE, STAINED WITH HÆMATOXYLIN
 $\times 50$.

- a.*—Tubules.
- b.*—Connective tissue.
- c.*—Ducts of tubules opening into,
- d.*—Rete testis.
- e.*—Capillary blood-vessels.

Fig. 144.



Fig. 145.



they are derived by kariokinetic division. The spermatoblasts form a broad zone of small granular nucleated cells, which show no signs of multiplication.

In many tubules spermatogenesis may be no further advanced than as indicated above; in many the lumen may be filled with spermatozoa, and others will show intermediate stages. These stages it will be well to consider in series. The first change that occurs is that the spermatoblasts (1 *c*) become somewhat oval in shape, and attached in the manner indicated in the figure to the elongated sustentacular cell (*d*). They become attached by one end of the oval containing the nucleus, while the other is directed towards the lumen of the tube. The gradual development of the spermatozoa from these cells is shown in the succeeding divisions of the figure, 2, 3, 4, 5. In (2) the oval cells are beginning to elongate, and their nuclei to be placed more definitely at their outer ends. In (3) the change is still more marked, and from the opposite end of the cell a cilium projects. The change is still further followed out in (4) and (5), in which it will be noted that the stalk of the sustentacular cell (*d*) is becoming lengthened and attenuated, so that the spermatozoa will ultimately be set free. While the first crop of spermatoblasts has thus become converted to spermatozoa, the cells of the intermediate layer (5 *b*) have produced a fresh one, ready to fix themselves upon the remains of the sustentacular stalk (or it may be upon a newly-developed sustentacular cell), when it has got rid of the first set of spermatozoa. The sustentacular cell has a chief mechanical function, and possibly a secondary nutritive one.

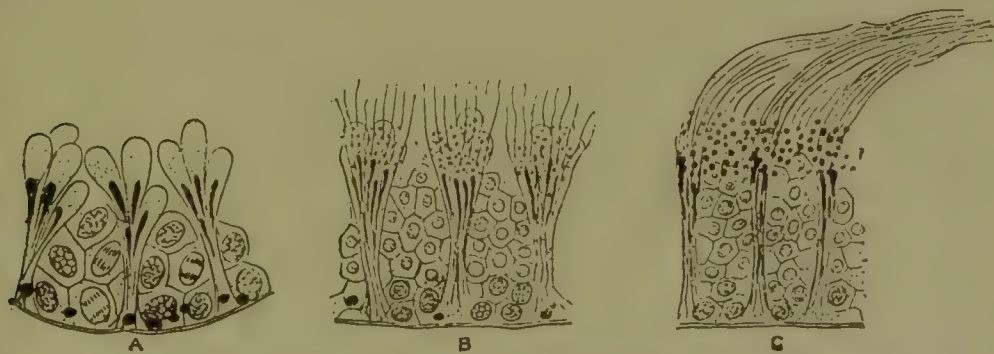


Fig. Q.—Sections of tubules of testis of rat, showing stages of spermatogenesis (semi-diagrammatic).

The different stages of development may frequently be seen in adjacent tubules of the same gland. Fig. 146 shows three tubules in section. In (A) we have the early stage of attachment

of the spermatoblasts to the sustentacular cells, and their commencing development; in (B) the tails have developed, but the heads are still arranged in "fan-shaped" groups, due to their attachment to the sustentacular cells; in (C) the spermatozoa form a continuous layer, their fan-like disposition having disappeared owing to the continued attenuation of their attachment to the sustentacular cells, or perhaps the solution of this connection altogether. *Fig. Q* represents the same three stages, but in a semi-diagrammatic way (see previous page).

In the development of a spermatozoön from a spermatoblast, the nucleus of the cell elongates and passes to one end. From the other the tail grows; it has its origin either from the protoplasm of the cell, or from the nucleus. Part of the protoplasm appears to disintegrate, and part forms the body of the spermatozoön.

Kariokinetic Changes in Spermatogenesis.—It appears that the spermatogenic cell of the second layer, when about to give rise to spermatoblasts, divides in the usual manner by indirect fission, in which splitting of the V-shaped loops (or chromosomes) occurs, to form two daughter cells of the *first generation*, in each of which the chromosomes are of the same number as in the parent cell. While still in the dyaster stage the chromosomes of the daughter cells themselves undergo splitting, to form a *second generation*, in which the number of the loops is still maintained. The cells of this generation again divide without reaching the resting stage, but this time without splitting of the chromosomes; so that the *third generation* possesses only half the number characteristic of the species. Thus if four is the number, the original spermatogenic cell acquires eight by splitting, and of these four go to one daughter cell and four to the other. Each of these daughter cells doubles its number of chromosomes by splitting, in the same way, so that the cells of the immediately succeeding generation (the second) still possess the species number, four. These cells, however, divide without splitting of their loops, so that the third generation of cells (those whose nuclei form the heads of the spermatozoa) contain only half the normal number. In the case of the ovum, the same process of reducing division is carried on, the original cell acquiring eight chromosomes by splitting, of which four go to the first polar body, and four remain in the ovum. The first polar body is extruded and lost, and the nucleus of the ovum divides without

further splitting of its chromosomes. Of this division the second polar body (also extruded) accounts for one half, while the other, consisting of two chromosomes, remains as the female pro-nucleus of the ovum. Thus, in an animal whose species number of chromosomes is four, the spermatozoön contains two, and fuses with the female pro-nucleus, which possesses a similar number. In this way the original number four is restored, and it is maintained in all subsequent cleavage by an accompanying splitting of the chromosomes.

This reduction of the chromosomes in connection with the development of sperm cells has been followed out in the case of *ascaris*, and also in the salamander, but it has not yet been demonstrated to hold good for mammals.

THE FEMALE ORGANS.

The Ovary.—The ovary consists for the most part of a stroma of fusiform connective tissue cells, containing in its substance ova in various stages of development, and enveloped by a layer of cubical or columnar granular cells. The ova are developed from this layer, which is hence called *germinal*. It is continuous with the rest of the epithelial lining of the peritoneal cavity, and surrounds the ovary except at its attachment to the broad ligament, where the blood-vessels and nerves enter it at the hilum.

The ova are developed at an early period in the life of the embryo from the germinal epithelium. Certain of the cells appear to be picked out as it were for the function, and each one so selected, surrounded by some of its fellows, sinks into the connective tissue beneath the surface, from which it becomes entirely cut off. The ovum cell now increases in size, exhibiting a well marked nucleus and nucleolus (or nucleoli), while the epithelial cells which have accompanied it arrange themselves round it, in a wholly subordinate capacity, to form a layer of cubical or columnar nucleated cells. In addition, the connective tissue in the immediate neighbourhood shows some sign of contributing a fibrous covering to the whole; and we have thus a *Graafian follicle*, as it is termed, in an early stage of development (*Fig. 150, b*). At this stage they exist in large numbers as a well marked layer beneath the epithelium covering the surface of the organ, but separated from it by the *tunica albuginea*, a special layer of stroma cells lying in the surface plane. This peripheral

FIG. 146.

S. TESTIS OF RAT, SHOWING STAGES OF SPERMATOGENESIS, STAINED
WITH HÆMATOXYLIN \times 400.

A, B, C.—Three stages of spermatogenesis.

d.—Connective tissue.

e.—Interstitial cells.

FIG. 147.

T.S. HUMAN EPIDIDYMIS, STAINED WITH HÆMATOXYLIN \times 300.

a.—Epithelial lining.

b.—Fibrous investment.

c.—Connective tissue between tubules.

d.—Blood-vessels.

Fig. 146.

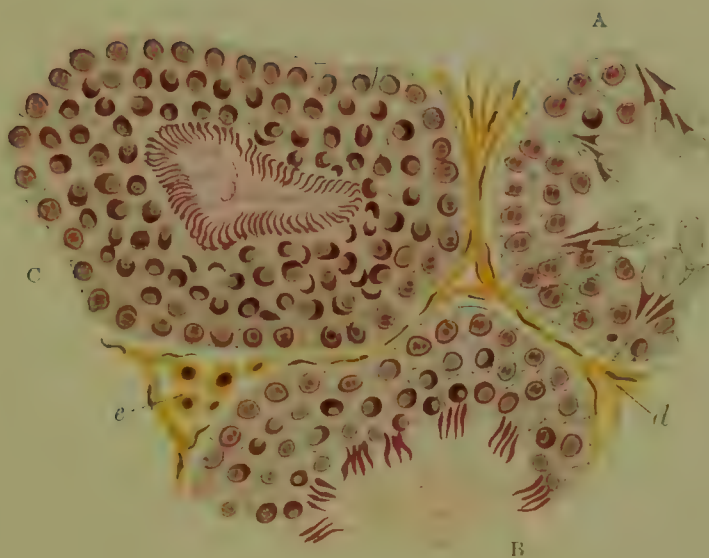
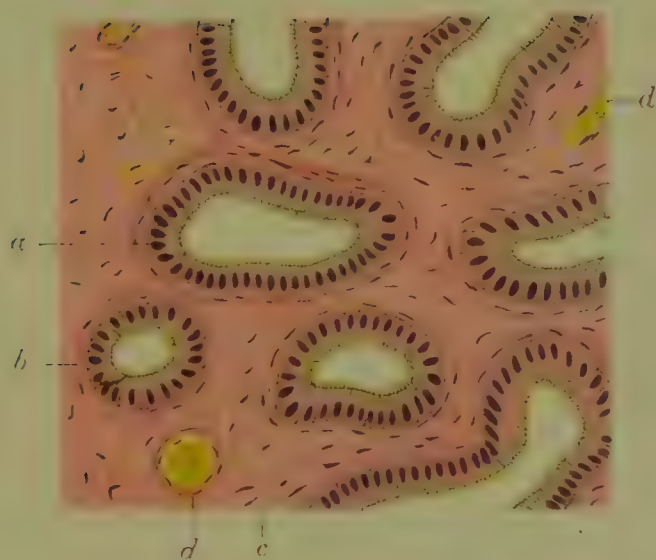


Fig. 147.



germinal zone of ova persists throughout life, and from it the supply of ova which are periodically cast off is drawn. When one of these is about to undergo further development, or "mature," or "ripen," as it is often called, it sinks from its peripheral position towards the centre of the organ, and the following further changes take place: The ovum, or cell itself, increases in size, and there appear in the meshes of its perinuclear protoplasm numerous granules of yelk substance, which increase in number with the growth of the cell. The nucleus shares also in the enlargement, and is now called the *germinal vesicle*, and its nucleolus the *germinal spot*. The single layer of cubical or columnar cells surrounding the ovum becomes increased to two, or it may be more, of which those lying internally and externally retain their original shape, while the intermediate cells, if any are present, are polygonal in outline. Between the epithelium and the ovum there appears a membrane, perhaps cuticular in nature, the *zona pellucida*, or, as it is also called, the *zona radiata*, from the fact that it exhibits radial striæ. These striæ have been said to represent channels containing processes from the surrounding epithelial cells, and to be associated with the introduction of food material to the perinuclear portion of the ovum or *yelk*. The spindle shaped cells of the stroma become arranged round the follicle to form a vascular *theca* or envelope, bearing on its inner surface a delicate structureless membrane, the *membrana propria*.

At this stage (*Fig. 150, c*) the follicle may be considered as half developed, and further changes take place before it is ready to be discharged. It continues to grow in size, and as it does so the epithelial cells surrounding the ovum increase in number, and finally show signs of separation into two layers by the appearance in their midst of the *liquor folliculi*. The one layer surrounds the ovum itself, and is termed the *tunica granulosa*, the other lines the *membrana propria*, and is called the *membrana granulosa*. But these two layers of cells are not completely separated from each other; they remain in contact at one point, which serves for the attachment of the ovum, which would otherwise float free in the *liquor folliculi*. The cellular projection resulting from this union of the two epithelial layers is termed the *cumulus*, or *discus proligerus*. As the ovum increases in size the fluid or *liquor folliculi* increases in quantity, and the whole follicle commences to return to the surface of the ovary,

from which it projects in the form of a bleb, the intervening tissues falling away to either side, as it were, as it advances. The wall of the bleb consists of the epithelium covering the whole organ, the attenuated tunica albuginea, the theca, and membrana granulosa of the Graafian follicle ; and at some point in it is situated the discus proligerus containing the ovum. When the bleb bursts, the ovum, surrounded by the cells of the tunica granulosa, escapes, and is received into the fimbriated extremity of the Fallopian tube, along which it is to pass towards the uterus.

After the ovum has escaped certain changes take place in the now empty follicle, resulting in the formation of a *corpus luteum*. The wall of the follicle has necessarily been ruptured, and this is frequently followed by the escape of blood from the broken vessels into the space itself. The cells of the membrana granulosa rapidly multiply, the membrane itself becoming much folded during its growth, so as to give in section the appearance of radiating plaits, and the appearance is rendered the more striking by the projection inwards of vascular septa of connective tissue as the result of proliferation of the stroma cells. So that at this stage the corpus luteum is a rounded body, composed largely of proliferated epithelial cells, traversed by radiating septa of connective tissue, the centre of the mass being frequently occupied by effused blood. The elements of the corpus now undergo degeneration, and the resulting fat becomes tinged with a pigment *lutein*, of somewhat uncertain nature, but in any case a derivative of the hæmoglobin of the blood. It is this yellow pigment which gives to the body the colour from which it derives its name. Finally, absorption of the degenerated mass takes place, and only a cicatrix is left. When pregnancy follows the discharge of an ovum, the resulting corpus luteum is much larger than on other occasions, and the time required for its absorption much longer.

The *stroma* of the ovary differs somewhat from ordinary connective tissue. It is composed of spindle shaped nucleated cells, without fibres between them, the latter being found only in the wedge of tissue entering with the blood-vessels at the hilum. Here, too, non-striped muscle cells occur. In some animals, *e.g.*, the rabbit and cat, a large number of nucleated, granular, polygonal cells (*Fig. 150, j*) are found amongst the ordinary spindle cells of the stroma. They are possibly the remains of

the cells of the hypertrophied membrana granulosa of previous corpora lutea, though this can hardly be if they correspond with somewhat similar cells found in the intertubular connective tissue of the testis. They do not occur outside the inner margin of the tunica albuginea.

The Fallopian Tube.—The Fallopian tube commences with a comparatively free fimbriated extremity, the lumen of which is in communication with the peritoneal cavity, and terminates in the angle of its own side at the upper end of the uterus. It lies in the edge of the broad ligament, and is thus for the most part of its circumference covered with peritoneum. Within this is a well marked muscular coat, consisting of a narrow external longitudinal, and a broad internal circular division; surrounding a mucosa of ordinary connective tissue, containing blood-vessels and lymphatics, and lined internally with a single layer of ciliated epithelial cells. The inner surface of the tube is not smooth but plicated in a very complicated manner, as may be seen in the transverse section shown in *Fig. 151*. No glands are found in the mucosa.

The Uterus.—The uterus in many animals, such as the rabbit and guinea-pig, appears to be formed by the union of the two Fallopian tubes to form one, and the resulting organ is not very much thicker than these are. In man, however, the uterus is very markedly thicker and larger than the tubes which open into it, and it possesses a more complicated structure. Its peritoneal investment encloses a well marked muscular coat, which forms the greater part of the substance of the organ. It may be considered to be composed of an outer thinner portion (which in itself appears to be in two divisions, an external longitudinal, and an internal circular or oblique), and an inner thicker one, in which the fibres run in various directions. The lines of separation between these different divisions, however, are not at all well marked, the strands of muscular fibres appearing as a rule to run in every direction. The inner thicker portion described above has been said to represent a hypertrophied muscularis mucosæ. In all parts of the muscular wall large veins with thickened walls are to be seen in considerable numbers, and form a very distinctive and characteristic feature of the tissue in this situation. The mucosa also is somewhat remarkable. Its basis is not formed of ordinary connective tissue, but of a special variety peculiar to the situation. It consists of a number of

small nucleated, often spindle-shaped cells, with little or no intercellular fibrillation, and is freely supplied with blood capillaries and lymphatics. It is lined internally with a layer of ciliated epithelial cells, which differ somewhat in character from those hitherto met with. They are smaller and more cubical, and their cilia are so delicate that unless special care is taken in preparing the tissue they cannot usually be made out in sections. The epithelium is prolonged outwards into the connective tissue of the mucosa in the form of the *uterine glands*, which are of the simple tubular variety, and consist of a tortuous tube with a large lumen, lined by ciliated cells, continuous with and similar to those lining the cavity of the uterus between them. The tube is for the most part a single one, but in some cases it forks at its lower extremity. There is no very sharp line of demarcation between the mucosa and the muscular coat, strands from the latter running up between the lower or outer ends of the glands, as shown in *Fig. 149*.

In the cervix the structure of the uterus becomes somewhat modified. The muscular fibres are more definitely arranged, and the mucosa becomes thrown into a number of ridges, which radiate from two main anterior and posterior longitudinal folds.

The glands also alter in character. The cells lining them lose their cilia, and the tube itself becomes shorter and branched. At the os uteri the single layer of cubical epithelial cells lining the lower part of the cervix passes suddenly into the stratified squamous epithelium of the vagina.

THE MAMMARY GLAND.

This is a gland of the compound racemose type, and consists in the human species of some twenty subdivisions, which are separated from each other by fibrous septa, and open by independent ducts upon the surface of the nipple. Each of these primary lobes is itself subdivided by connective tissue partitions into secondary lobes, and these again into lobules. Each of the twenty original ducts subdivides again and again in the usual manner, as it distributes itself to the ultimate alveoli of the gland. These are somewhat irregular in shape on section, tending on the whole to be round, and are lined by an epithelium, which varies in character at different times. When the gland is secreting milk (*i.e.*, during the period of lactation) the cells are

tall and somewhat columnar in shape, with a conical internal apex and a broader external base, and project in varying degrees into the large lumen, which thus presents a more or less irregular outline. It is filled with fat globules of various sizes, such as are always found in milk, and it also contains occasional fragments of epithelial cells. The tall cells projecting into it have very distinctive characters. They possess a well marked nucleus near their base, which rests upon a *membrana propria*; in the inner portion of the cells fat globules may be seen of various sizes, sometimes projecting into the alveolar lumen, into which they are on the point of being released. In many cases the cells show an additional nucleus in their inner part, and this fre-

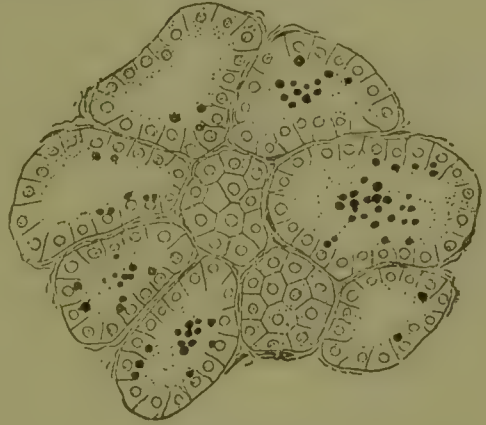


Fig. R.—S. Mammary gland of mouse, stained with osmic acid x 300.

quently becomes detached, together with a certain part of the protoplasm and the cell wall, and floats free in the lumen together with the secreted fat globules. So that by the detachment of the central ends of the columnar cells a still more jagged contour to the lumen may be given. The above description represents the typically active condition of an alveolus of the mammary gland during lactation, but it must be noted that all the alveoli are not necessarily secreting at the same time, some of them being in the quiescent state, which is characteristic of the alveoli between two periods of lactation. During the period of rest the alveoli present a very different appearance from that already described. Their lumen is larger, it is empty, and surrounded by cells which have entirely lost their columnar or conical shape, and become cubical or even flattened. They are simply cubical, nucleated, granular cells, somewhat similar to those lining the alveoli of an undistended foetal lung, and, in fact, it is not unusual to speak of this condition of the mammary gland as the "lung stage." The third condition to be mentioned is that which obtains previous to lactation, *i.e.*, in an individual in which pregnancy has not yet occurred. Here the alveoli consist of solid columns or masses of small polyhedral nucleated cells, which are thus not arranged round a central lumen. When lactation ensues on pregnancy the central cells degenerate, and are carried away as *colostrum cor-*

FIG. 148.

SPERMATOOA, STAINED WITH HÆMATOXYLIN, HIGHLY MAGNIFIED.

- a.*—Spermatozoön of guinea-pig, with part of protoplasm of spermatoblast still attached.
b.— " " seen from the side.
c.—Spermatozoön of horse.
d.— " newt.

FIG. 149.

S. HUMAN UTERUS, STAINED WITH HÆMATOXYLIN × 200.

A.—Mucosa.

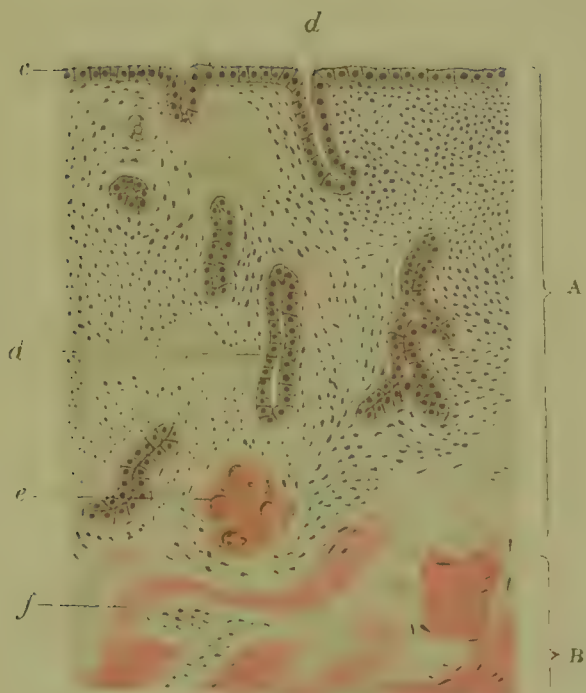
B.—Muscular substance.

- c.*—Lining of ciliated epithelium.
d.—Section of glands.
e.—Blood-vessels.
f.—Muscular strands.

Fig. 148.



Fig. 149.



puscles in the first milk, leaving a single permanent layer lining the alveoli to carry on the secretion.

The main ducts of the gland are lined by cubical cells, but the epithelium becomes flattened in the ductules, which frequently present enlargements or sinuses upon them within the lobules. The main ducts also, immediately prior to opening on the nipple, each exhibit a dilatation.

Examine the following specimens :—

(1.) *S. Testis of cat or rabbit, stained with hæmatoxylin. B. (Fig. 145 ; see also Fig. 144.)*

Use the testis of a cat or rabbit, cut so as to show the rete and epididymis, for a low power view.

Note the tunica albuginea (*Fig. 144, a*) surrounding the organ and fusing with the connective tissue, entering it at the back to form the corpus Highmorianum. Trace the tissue forwards in the gland, and note that it breaks up to form the septa (*e*), which extend radially till they join the inner surface of the tunica albuginea. Now note the position of the epididymis (*i*). It is placed at the back of the testis, outside it, behind the corpus Highmorianum, and can readily be seen under this power to consist (in section) of a number of tubes lined with epithelium cut transversely. Now look at the tubuli seminiferi in the compartments marked off by the fibrous septa in the testis itself. They seem to have little or no lumen, and to be composed of a somewhat granular looking mass of cells. In many tubules look for somewhat fan-shaped collections of deeply stained elongated nuclei (the heads of the developing spermatozoa) arranged in a radial manner round the centre. Now follow the tubes along the tubuli recti (*f*) till the rete testis (*g*) is reached, and note its character. This is better shown in *Fig. 145, d*. It can be seen to be lined with a layer of flattened cells, and to form a somewhat intricate network. In some cases the network is not very clearly revealed in sections, and appears more as an irregular fissure. The connection between the rete and the epididymis is not shown in most sections, the latter appearing quite as a separate organ with a distinct band of connective tissue dividing it from the testis itself.

Under the high power examine first the tubules of the testis. Note the distinct basement membrane (often in more than one layer) by which they are surrounded, and observe that it is

formed of one or more thin laminae of the intertubular connective tissue. The tubules themselves will be seen to be in different stages of spermatogenesis, and the student should make these out as far as possible; but they will be better seen in the following specimen, which is specially intended for examination under the high power. Look for the transition (in the more central tubules) between the several layers of cells and the single layer lining the tubuli recti continuous with them. The tubuli recti may be regarded as corresponding with the intermediate ducts of a salivary gland. Note the loose character of the intertubular connective tissue, containing blood-vessels, lymphatics, and nerves, and in some animals a large number of interstitial cells. These are yellow, polygonal, granular and nucleated, and may be either modified connective tissue cells, similar to the plasma cells of Waldeyer, or they may be epithelial in nature, and represent the remains of the Wolffian body. Compare them with the interstitial cells found in the stroma of the ovary, to which they are very similar. Now examine the epididymis cut transversely. The tubules are enveloped in a sheath of connective tissue, and are characterised by their very wide lumen, which may contain a confused mass of spermatozoa. Note the single layer of tall columnar cells with long cilia lining them (*Fig. 147*). Look for a transverse section of the vas deferens, which, if present, will be seen a little apart from the epididymis. Note its two well marked muscular coats, and its mucosa lined with columnar non-ciliated epithelium.

(2.) *S. Testis of rat, stained with saffranin, hæmatoxylin, or borax-carmine (for spermatogenesis). (Fig. 146.)*

This should be a particularly thin specimen, and is intended especially for the study of the changes taking place in the cells lining the tubules. Look for all the stages shown in the diagrammatic figure (*P*, p. 441). In some tubules, as shown in (5), two phases may be seen, where the first crop of spermatozoa are not yet discharged, but a new generation of spermatoblasts is ready to take their place when this happens. Examine first one tubule and then another with the draw-tube out and a lens magnifying 600 or 700 diameters. It is well to become conversant with the theory of spermatogenesis before examining the specimen, and then the recognition of the three layers of cells in the tubules, *viz.*, (1) the spermatogonia, (2) the spermatogenic cells, and (3) the spermatoblasts, presents no great difficulty; the spermatozoa, either

as fan-shaped, deeply stained groups, or when forming a complete ring, previous to discharge, are always readily recognisable. But without a clear idea of the theory an examination of the specimen in this case yields little information.

(3.) *Spermatozoa of small mammal, unstained.*

Incise the epididymis of an animal recently killed, smear a little of the milky fluid on a slide, and examine under the high power. Note the spermatozoa with their refractile heads and actively moving vibratile tails. Allow a thin layer of the fluid to dry on a slide; cover, and "ring" with gummed paper. Or make a cover-glass preparation by sliding one cover over another with a small drop of the fluid between them; allow to dry, and stain in methyl-blue, etc.

(4.) *S. Ovary of cat or rabbit, stained with hæmatoxylin. B. (Fig. 150.)*

Under the low power observe first the narrow layer of columnar epithelium surrounding the section; its more intimate structure will be better seen under the high power. Observe the cellular character of the stroma of the organ, and the position of the ova in their earlier and later stages of development. The young ova, round, and more or less crowded together, may be seen beneath the epithelial covering, the tunica albuginea intervening; the larger ones, of which half-a-dozen in different stages may be seen in the same specimen, have sunk to a varying extent towards the centre. Look for two stages of development especially; one in which division of the epithelium, surrounding the ovum, into two layers by the liquor folliculi, has not yet commenced (*c*), and another in which it is well advanced (*d*). Note the position of these ova in the specimen, as they will require examination under the high power. With this power find again the surface epithelium (*a*), and note the cubical or columnar character of its cells, which thus differ from the rest of the peritoneal epithelium, which is squamous. Now study the peculiar nature of the ovarian stroma, or connective tissue basis of the organ. It is not composed of ordinary connective tissue, but of spindle shaped cells, which are apparently not separated from each other by any fibrillated fibres. Look for numerous blood-vessels cut in different directions, containing blood corpuscles, which are of a greenish yellow colour, and also note the presence of many interstitial cells (*j*). Observe that these cells do not usually occur in the peripheral part of the stroma, which

FIG. 150.

S. OVARY OF RABBIT, STAINED WITH HÆMATOXYLIN $\times 250$.

- a.*—Germinal epithelium.
- b.*—Small ova.
- c.*—Ova in intermediate stage of development.
- d.*—Graafian follicle (membrana granulosa of).
 - e.*—Tunica granulosa.
 - f.*—Zona pellucida.
 - g.*—Yolk.
 - h.*—Germinal vesicle.
 - i.*—Discus proligerus.
- j.*—Interstitial cells.

FIG. 151.

T.S. FALLOPIAN TUBE OF PIG, STAINED WITH PICRO-CARMINE $\times 100$.

- a.*—Mucosa.
- b.*—Muscular coat

{	<i>d.</i> —External division, longitudinal. <i>e.</i> —Internal ,, circular.
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- c.*—Peritoneum.
- f.*—Subepithelial connective tissue.

Fig. 150.

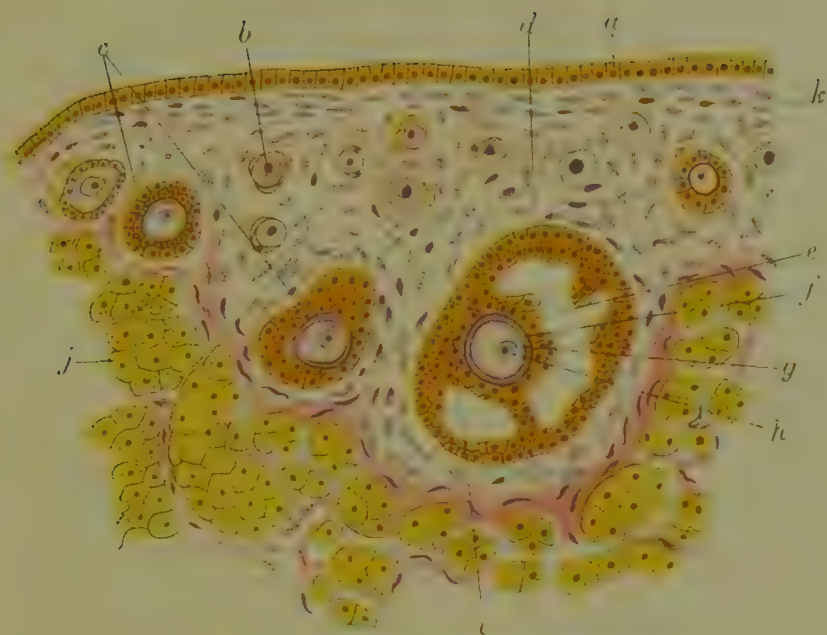
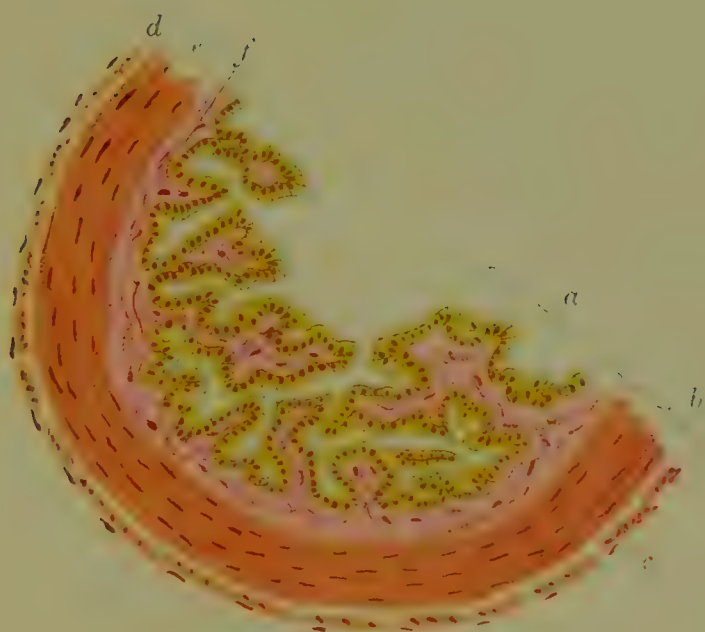


Fig. 151.



contains the row of young ova. Towards the centre of the ovary look for a certain amount of ordinary connective tissue, together with fibres of non-striped muscle, which have entered the organ with the blood-vessels at the hilum. Return now to the epithelial investment, and note that the stroma immediately beneath it is condensed to form a special band (in section), the tunica albuginea, the fibre cells of which run in the surface plane. Within this observe the small ova—round, nucleated cells surrounded with a single layer of cubical epithelium; and then pass to those of a rather larger size (*c*), placed usually a little further inwards. In these the surrounding epithelium is represented by a double row of well marked cubical or columnar cells, and the ovum itself has become invested with the zona pellucida; its perinuclear protoplasm now forming the yelk, its nucleus the germinal vesicle, and its nucleolus the germinal spot. Observe, too, that the stroma immediately in contact with the whole is becoming condensed to form a definite *theca*. Now find one of the larger ones still more deeply placed (*d*), and note the multiplication of its investing epithelial cells, and their division by the liquor folliculi into two layers, an outer the membrana granulosa (*d*), and an inner the tunica granulosa (*e*), the two being fused at one point, the discus proligerus (*i*). But in some cases, as shown in the figure, they touch at more than one point, the separation into two layers being incomplete. Note the increased distinctness of the zona pellucida (*f*), the development of the yelk (*g*), and the germinal vesicle (*h*). Lastly, if present, find a corpus luteum. These vary in appearance very much with their age, and also according to whether pregnancy followed the extrusion of the ovum. They are usually large, round bodies of a yellow colour, and present a radiating appearance, due to the plication of the theca, and the presence of septa of connective tissue running in between the folds towards the centre of the mass.

(5.) *T. S. Fallopian tube of pig, stained with picro-carmin.* F.
(Fig. 151.)

Under the low power examine the general arrangement of the parts of the section, noting the mucosa (*a*), and the two divisions of the muscular coat (*b*). Observe the very irregular lumen of the tube, due to the folding of the mucous layer. This layer is, in fact, so much folded that the folds almost touch each other in the centre, the lumen being merely the fissure between their opposing surfaces. There are no glands, only the crypts between

adjacent folds. Under the high power observe especially the character of the epithelium, which is ciliated and in one layer. Look also for indications of a peritoneal investment outside the muscular coat.

(6,) *T.S. Uterus of young girl, stained with hæmatoxylin. B. (Fig. 149.)*

Under the low power note the narrow mucosa, of a granular, blue appearance (in contrast to the fibrous, pinker, muscular coat), immediately surrounding the slit representing the lumen of the organ. Distinguish the line of epithelium internally with its deeply stained nuclei, and the glands dipping down from it into the mucosa. Note now the very broad muscular coat, the fibres of which seem to run in every direction. Observe the blood-vessels which are very much in evidence from the thickness of their walls ; they are quite characteristic of the uterus. Under the high power examine the mucosa as shown in the figure. Find the lining epithelium (*c*), and identify the cubical or columnar character of its single layer of cells, and endeavour to distinguish the cilia. These are not always to be seen, as they are very delicate and liable to be lost in the process of preparation. Trace the epithelium down into the mucosa, where it is continued in the form of gland tubes (*d*), which will be seen cut in various directions, and sometimes branching at their lower or outer extremities. Study the substance of the mucosa itself, and note its "small-celled" character, and freedom from ordinary fibres. Observe the numerous small blood-vessels, and in its lower or outer part strands of non-striped muscle projecting into it from the muscular coat. Examine the latter, and note the very complicated interlacement of the strands of muscle fibres. The distinction into internal and external division is scarcely to be made out definitely. Externally there is the serous coat continuous with the peritoneum, but the epithelium cannot always be differentiated.

APPENDIX TO CHAPTER XV.

METHODS OF PREPARATION.

1. Testis of cat, rabbit, or guinea-pig (for low power view).—Incise the gland, harden in Müller and spirit, cut in gum, stain in hæmatoxylin, and mount in balsam ; or stain in bulk in borax-carmines, and cut in paraffin.

2. Testis of rat or guinea-pig (for spermatogenesis).—Harden small pieces in Flemming's fluid, cut in paraffin, and stain in saffranin ; or harden small pieces in osmic acid (1 per cent.) for twenty-four hours, complete in alcohol, stain in bulk in Kleinenberg's hæmatoxylin or borax-carmines, and cut in paraffin. The different phases may frequently be beautifully brought out in this way.

3. Epididymis (human).—Obtain a piece of human epididymis as fresh as possible, harden in Müller and spirit, cut in gum, stain in hæmatoxylin, and mount in Farrant or balsam ; or stain in bulk in borax-carmines, and cut in paraffin. The long ciliated cells of the tubules of the epididymis are very beautifully seen in that of man. The vas deferens may also be prepared in the same way.

4. Ovary of cat, rabbit, or guinea-pig.—The ovary may be hardened in Müller and spirit, or in Kleinenberg's picric acid solution ; stained in bulk in borax-carmines, or Kleinenberg's hæmatoxylin, and cut in paraffin. It may also, if desired, be cut in gum, and subsequently stained, but the ova of the fully developed Graafian follicles very readily fall out of position.

5. Fallopian tube of small mammal.—Harden in Müller and spirit, stain in bulk, and cut in paraffin ; or cut in gum, and stain subsequently as usual.

6. Uterus of child.—Harden in Müller's fluid, cut in gum, stain in hæmatoxylin, and mount in balsam. If a suitable uterus cannot be got sufficiently fresh, use that of a dog or cat. Harden in Müller and spirit, cut in gum, and stain subsequently ; or stain in bulk, and cut in paraffin. In preparing the human uterus it is better to use Müller's fluid alone without the spirit, as the organ tends to become too hard to cut well.

7. Mammary gland.—Use the gland of a pregnant cat or rabbit. Harden in Müller's fluid, cut in gum, and stain with picro-carmines and osmic acid, to show the fat globules. Avoid the use of spirit in hardening, as the fat is then dissolved. Or harden the gland (small pieces) in Flemming's fluid, cut in paraffin, and stain in saffranin ; or stain in bulk in borax-carmines, and cut in paraffin.

CHAPTER XVI.

THE EYE, EAR, AND OLFACTORY MEMBRANE.

THE EYE.

The Structure of the Eye.—The following are the chief parts to be considered :—

1. **The Sclerotic, Cornea, and Conjunctiva.**—The sclerotic is the outermost coat of the eyeball. It is thick, tough, and inelastic, and gives support and protection to all the structures enclosed within it. It is continuous posteriorly with the dura mater investing the optic nerve, and anteriorly with the transparent cornea. The conjunctiva extends upon the surface of the sclerotic from the margin of the cornea till it is reflected upon the inner surface of the eyelids as the palpebral layer to join the general skin surface at their edges. Only the epithelium of the conjunctiva is continued over the surface of the cornea, of which it forms the anterior epithelial layer.

2. **The Choroid, Ciliary Processes, and Iris.**—The choroid is vascular, pigmented, and elastic, and corresponds with the pia mater of the brain. Between it and the sclerotic is a lymph space, or system of lymph spaces, corresponding with the arachnoid. About the point of junction of the sclerotic with the cornea, the choroidal coat greatly thickens, becoming at the same time largely composed of white, fibrous tissue. It here gives off the ciliary processes on its inner side, and is continued, separated, however, from the cornea, as the iris towards the more central part of the globe. But, whereas the cornea completes the sclerotic investment anteriorly, the iris leaves that of the choroid deficient in the region of the pupil, and thus forms a perforated screen between the light in front, and the general cavity of the optic cup behind. At the junction of the cornea and sclerotic is also to be found the ciliary muscle, which passes backwards to be inserted

into the anterior margin of the choroid coat. In the iris itself are strands of muscle running in two directions—(*a*,) circularly, the sphincter pupillæ; (*b*,) radially, the dilator pupillæ.

3. The Optic Nerve, Retina, and Pars Ciliaris Retinæ.—The retina is the innermost of the three coats of the eye, and is essentially nervous in origin and function. It may be regarded as an outlying part of the brain, continuous with the more central portion through the optic nerve. It develops as a hollow outgrowth from the first cerebral vesicle, subsequently becoming invaginated to form the optic cup. The sclerotic and choroid are formed around it from the mesoblast; and the blood-vessels of the retina, the vitreous humour, and the suspensory ligament, have the same origin.

The optic cup, like other invaginations, consists of an inner and an outer part. In the developed eye the inner wall is represented by the retina (proper), the pars ciliaris retinæ, and the epithelium (pigmented) continuous with it at the back of the iris; the outer wall by the pigmentary layer of the retina, and its continuation in a modified form over the ciliary processes and the back of the iris. Thus, at the back of the iris, each wall consists of a layer of pigmented cells; over the ciliary processes the inner is represented by a layer of unpigmented columnar or cubical cells (the ciliary part of the retina) resting on the outer, a layer of pigment cells; in the posterior three-fourths of the cup, the inner wall is the retina itself, the outer, the layer of hexagonal pigment cells.

The optic nerve passes through the sclerotic, the choroid, and the pigmentary layer of the retina, at the back of the eyeball, and expands immediately into the inner retinal cup or retina proper, which extends forwards till it becomes reduced to the pars ciliaris retinæ at the ora serrata, a little posterior to the ciliary processes. Thus, though the optic cup may be considered to extend from the margin of the pupil in front to the entrance of the optic nerve posteriorly; the retina, ceasing abruptly at the ora serrata, only corresponds with the posterior three-fourths of it.

4. The Lens, Vitreous Humour, and Suspensory Ligament.—The lens, like the cornea, is translucent. It is derived from the epiblast, a portion of which becomes enclosed in the optic cup as a hollow bud, at an early stage of the development of the eye. It lies immediately behind the iris, and is retained in position by the suspensory ligament, which connects it to the

ciliary processes and pars ciliaris retinae. The vitreous humour fills the space between the lens in front and the main cavity of the eyeball behind. It is developed like the suspensory ligament, and the blood-vessels of the retina, from mesoblastic tissue enclosed in the optic cup.

5. The Blood-vessels and Lymphatics of the Eye.—These, as well as the structures mentioned above, will be considered immediately in detail. It may be mentioned here that the general cavity of the eye may be regarded as being divided into three large lymph spaces. The first of these is the anterior chamber bounded in front by the cornea, and behind by the iris and lens; it contains the aqueous humour, and is a continuation of the lymph channels between the sclerotic and choroid coats. The second, or posterior chamber, is bounded by the iris in front, the suspensory ligament behind, and the ciliary processes to the outside; it is in communication with the anterior chamber. The third is constituted by the whole of the cavity of the optic cup behind the lens, and contains the vitreous humour.

The more minute structure of the different parts of the eye may now be considered.

1. The Sclerotic consists of a dense feltwork or interlacement of bundles of white fibres. Many of them are disposed longitudinally, *i.e.*, run in the sagittal line, while others pass between them from side to side, *i.e.*, in the coronal line, so that they cross each other at right angles. Ordinary connective tissue cells, many of which contain pigment, are scattered amongst the bundles, and there is a considerable number of small lymph spaces lined with epithelioid cells. There is a small blood supply. Towards the inner surface of the sclerotic the flattened pigment cells, seen as somewhat fusiform bodies in vertical section, are collected together in greater number, and to this innermost layer the term *lamina fusca* has been applied.

The sclerotic coat extends over the posterior five-sixths of the eye, roughly speaking. The anterior sixth is completed by the **cornea**, with which it is continuous, and which may be considered a special modification of it. Whereas the sclerotic is opaque and white, the cornea is translucent and colourless. It has the following structure: The main part is composed of a number of superimposed lamellæ of white fibrous, connective tissue, between which are found flattened *corneal corpuscles*—

ordinary connective tissue cells, which here assume a special form. They are flattened conformably with the surfaces of the lamellæ between which they lie, and possess a large nucleus. They have many characteristic, stiff-looking processes, sometimes branched, which anastomose chiefly with those of cells in the same plane, *i.e.*, between the same lamellæ, and to a less extent with the processes of cells in adjacent planes. The corneal corpuscles occupy cell spaces—comparable with those occurring in tendon—in the cement substance which unites the fibrils, fibres, and lamellæ together, and forms a general matrix for the tissue.

This fibrous basis of the cornea is covered on its anterior surface with a layer of epithelium, continuous at the margin with that of the conjunctiva. The epithelium rests upon a distinct basement membrane, and is of the stratified, squamous variety. It differs, however, from the epidermis of the skin and the hard palate, approaching more nearly the transitional epithelium of the bladder in character. There are comparatively few layers of cells—five, six, or seven—and they are not, as in the epidermis, divisible into corneous and Malpighian strata. The deepest or germinal layer consists, as elsewhere, of small, somewhat columnar cells, placed vertically on the basement membrane; above are two or three layers of somewhat pear-shaped or polygonal cells; at the surface the cells are inclined to be flattened, but do not form squames. All the cells retain their nuclei. The posterior surface of the connective tissue basis of the cornea is covered by a conspicuous elastic sheet—the *posterior elastic lamina*, or *membrane of Descemet*. It presents a very distinct double contour, and in many animals attains considerable thickness. It stains a bright yellow with picric acid. It is in its turn covered by a layer of flattened epithelial cells continuous with those lining the rest of the anterior chamber of the eye. There are thus four layers to be distinguished in the cornea from before backwards: (*a*,) the anterior epithelium resting on the basement membrane; (*b*,) the connective tissue or fibrous basis; (*c*,) the posterior elastic lamina; (*d*,) the epithelium of the anterior chamber.

The cornea contains no lymphatics or blood-vessels, thus differing from the sclerotic. The nutrition of the tissue is carried on by percolation of lymph from the blood-vessels at the periphery through the system of inter-communicating cell spaces containing the corneal corpuscles.

The Conjunctiva consists of a somewhat thin layer of stratified squamous epithelium, with subjacent connective tissue corresponding with the cutis vera of the skin, with which it is continuous at the margin of the eyelids. From the inner surface of the eyelid the conjunctiva is reflected upon the anterior part of the eyeball, where it forms the smooth, glistening surface to the sclerotic, commonly known as the white of the eye. As a distinct membrane, it ceases at the margin of the cornea, at which point the fibrous tissue disappears, the epithelium alone passing over the body of the cornea to form its anterior epithelial layer.

2. The Choroid is the vascular coat of the eye, and is the thinnest of the three. It consists of a peculiar tissue apparently composed of elastic fibres and pigmented connective tissue cells imbedded in a homogeneous matrix. In it are found blood-vessels surrounded by lymphatic sheaths (peri-vascular lymphatics), and lymph spaces lined with epithelioid cells are abundant. It may be described as consisting of three layers: the outermost, which is separated from the sclerotic by a large lymph space—the perichoroidal—is composed of several layers of elastic fibres and pigment cells in a homogeneous matrix, arranged in a lamellar manner, the lamellæ being separated from each other by lymphatic spaces lined with epithelioid cells. This portion of the choroid is termed the *lamina supra-choroidea*. The middle layer, or *choroid proper*, contains the large arteries and veins; the tissue between them consists of elastic fibres and pigment cells in a homogeneous matrix, and contains numerous lymphatic spaces in addition to peri-vascular lymphatics surrounding the blood-vessels. The internal layer, a much narrower one, is termed the *chorio-capillary coat*, and consists chiefly of a close network of the capillary blood-vessels into which the vessels of the middle layer break up. There is little tissue between the capillaries, and the pigment cells are less numerous or absent. Lining the inner surface of the choroid, and separating it from the retina, is a homogeneous membrane—the *membrane of Bruch*.

The sclerotic and choroid are, as we have stated, separated from each other by the perichoroidal lymph space throughout nearly their whole extent, and they consequently come apart with great readiness. At two points, however, they are closely attached to each other, viz., the ciliary region, and around the entrance of the optic nerve at the back of the eye. At this point

the sclerotic becomes continuous with the dura, and the choroid with the pia, and bundles of tissue from each pass into and across the "neck" of the optic nerve, forming the *lamina cribrosa*.

The Ciliary Processes.—At about the level of the ora serrata, the choroid coat begins to change considerably in character. It becomes increasingly thicker, and the inner surface is thrown into a series of radiating plaits—the ciliary processes. Anterior to these processes the vascular coat is known as the iris. The ciliary processes and the thickened continuation of the choroid, from which they project, differ from the thinner membrane behind in the replacement of the elastic fibres to a great extent by ordinary white fibrous tissue. The capillary blood-vessels, too, are not collected into a special chorio-capillary layer, but are diffused more evenly throughout the tissue. The membrane of Bruch, which we saw separated the choroid from the retina, is continued over the ciliary processes, and here separates their connective tissue basis from a layer of somewhat cubical pigmented cells—the continuation forwards of the pigmentary layer of the retina. Upon the pigment cells is placed a layer of clear, columnar, nucleated cells—the *pars ciliaris retinæ*, into which the retina itself becomes resolved at the line of the ora serrata. Many of these cells, especially those in the furrows between the plaits, are drawn out at their free extremities into fine tapering processes, with which the fibres of the suspensory ligament are continuous. On the outer side of the ciliary processes and lying in close relation with the sclerotic, is the ciliary muscle, and further forwards the spaces of Fontana and the pectinate ligament, but we shall return to these when the junction of the cornea, the sclerotic, and the iris is considered.

The Iris has the following structure from before backwards. Anteriorly it is covered by a single layer of flattened cells continuous with those at the back of the cornea; behind this is a layer composed of ordinary connective tissue, and containing few blood-vessels. The main body of the iris posterior to this contains large blood-vessels separated by connective tissue. Pigment cells occur in both layers, but in larger number in the last. The remaining layers are somewhat difficult to define, and different opinions have been entertained respecting their nature. It seems that the membrane of Bruch thins away upon the posterior surface of the connective tissue body of the iris, after it

FIG. 152.

S. HUMAN EYELID, STAINED WITH HÆMATOXYLIN AND EOSIN $\times 20$.

A.—Anterior surface of eyelid.

B.—Posterior (conjunctival) surface.

a.—Eyelash.

b.—Orbicularis muscle.

c.—Duct of one of the,

d.—Meibomian glands.

e.—Ciliary portion of orbicularis (circular muscle of Riolan).

f.—Glands of Moll.

FIG. 153.

ANTERO-POSTERIOR SECTION OF EYEBALL (DIAGRAMMATIC).

a.—Retina.

b.—Choroid.

c.—Sclerotic.

d.—Optic nerve.

e.—Macula lutea.

f.—Vitreous humour.

g.—Termination of retina anteriorly—ora serrata.

h.—Ciliary enlargement.

i.—Iris.

j.—Cornea.

k.—Anterior chamber of the eye.

l.—Lens.

m.—Posterior chamber of the eye.

n.—Suspensory ligament.

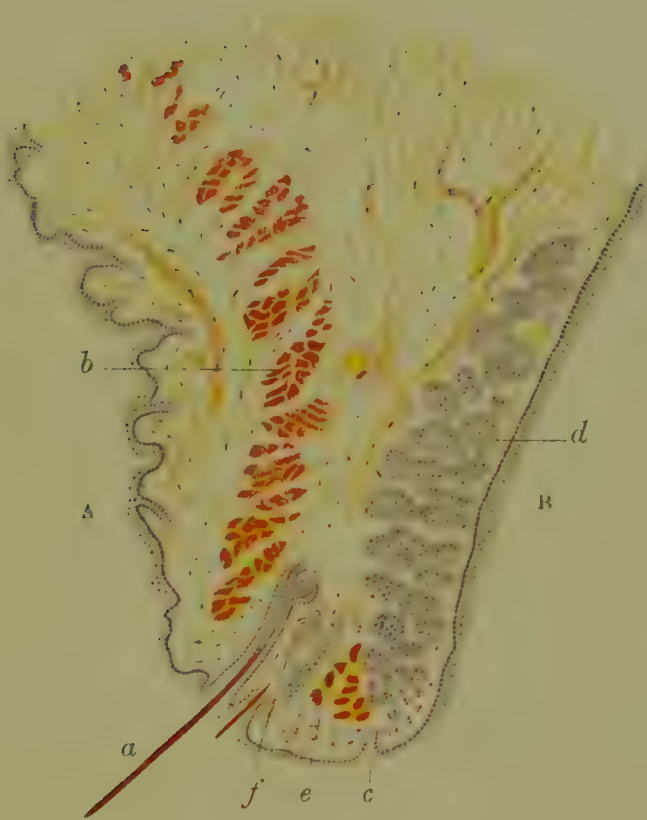


Fig. 153.



is continued forwards from the ciliary processes, and finally becomes indistinguishable. Upon this membrane, or if it is absent, upon the surface of the fibrous tissue, is a layer of pigment cells, cubical in form, continuous with those covering the ciliary processes, and, like them, corresponding with the pigmentary layer of the retina. So that with this layer, the parts in the developed eye representing the outer wall of the original optic cup are complete. The succeeding layer at the back of the iris, the most posterior one, is the continuation forwards of the pars ciliaris retinæ, and consists, not as one might anticipate, of clear cells, cubical or squamous in character, but of pigmented cells similar to those beneath. The two layers are so fused together that the outlines of their constituent cells are not easily distinguished, and they appear in vertical section as a dark band of pigment, with a somewhat irregular margin, which sometimes goes by the name of the *uvea*. The uvea terminates abruptly at the margin of the pupil, where the superficial layer of pigment cells becomes continuous with the flattened epithelium lining the anterior chamber of the eye.

The iris also contains fibres of non-striped muscle. Some of these are arranged circularly round the opening of the pupil, and constitute the *sphincter pupillæ*. Others, the existence of which is however questioned, are said to run radially outwards and to lie between the membrane of Bruch and the fibro-vascular body of the iris. These have been termed the *dilator pupillæ*.

The junction of the Cornea, Sclerotic, and Iris.—The iris, when followed outwards from the pupil, passes into the enlargement of the vascular choroid coat bearing the ciliary processes. But the iris has another very important connection, as part of its connective tissue substance is continued, not in a solid sheet, but frayed out into finger-like processes, into the point of junction of the cornea and the sclerotic. These processes are termed collectively the *pectinate*, or comb-like *ligament*. The bundles of white, fibrous tissue, of which the divisions are composed, pass amongst the connective tissue bundles of the sclerotic, and there become lost as separate structures. The junction of the iris with the cornea, by means of the *ligamentum pectinatum*, constitutes what is termed the *iridic angle*, limiting the anterior chamber externally. The clefts between the divisions of the ligament open into a network of lymph spaces immediately external to it, in the connective tissue of the ciliary enlargement. The spaces, which are

separated from each other by delicate fibrous trabeculæ, are termed the *spaces of Fontana*. Anterior, or external to these spaces, and situated in the peripheral part of the cornea, is a large lymph space lined with a layer of epithelioid plates—the *canal of Schlemm*—which is directly connected both with these and with the branches of the anterior ciliary veins. The aqueous humour of the anterior chamber is thus, through the spaces of Fontana, in communication with the venous system, and in the same manner with the lymphatic spaces of the ciliary enlargement and choroid coat behind it.

The epithelium of the posterior surface of the cornea is reflected over the bars of the pectinate ligament, and, folding in between them, covers the trabeculæ of connective tissue, separating from each other the spaces of Fontana. Followed backwards from the ligamentum pectinatum, it becomes reflected again upon the anterior surface of the iris, and joins the posterior layer of pigment epithelium, as already stated, at the margin of the pupil.

The posterior elastic lamina of the cornea is frequently stated to follow a somewhat similar course to the epithelium covering it, but it appears to be doubtful if it does so. It seems rather to end with more or less abruptness at the margin of the cornea, extending, however, far enough to be pierced by the divisions of the pectinate ligament. Immediately to the outside of the entrance of the ligament it rapidly thins, and (in section) comes to a point. It does not appear to be reflected to any marked extent upon the divisions of the ligament itself, and certainly never upon the anterior surface of the iris. *Fig. 156* shows a section of the cornea and posterior elastic lamina of the ox pierced by the ligament as seen from the anterior chamber, and the small extent to which the lamina is reflected from this point of view. The same is equally easily demonstrated in sections of the corneo-sclerotic junction made in the antero-posterior axis of the eye.

The ciliary muscle is of great importance in accommodation. It consists of non-striped fibres, and may be said to be attached to the inner surface of the junction of the cornea and sclerotic immediately behind the entrance of the pectinate ligament, and to extend backwards in a radial manner to be inserted into the anterior part of the choroid. It is somewhat fan-shaped, the angle of the fan corresponding with its point of origin, and the

broad end with its insertion. Some of the most internal of the fibres curve round so as to have a circular rather than a radiating direction, and these are sufficiently distinct in some animals to have been described as a separate circular muscle.

3. The Optic Nerve differs in many ways from an ordinary nerve. In the first place it is invested with an external sheath of dura mater, separated by an arachnoid space from a pial sheath, which lies immediately upon the nerve bundles, and sends inwards a network of septa between them. The bundles or fasciculi are not, however, as in an ordinary nerve, cylindrical, but in transverse section, polygonal or irregular, and are not surrounded by a laminated perineurium. Near to the eyeball the retinal artery and vein, surrounded by pial connective tissue continuous with the inter-fascicular septa, are found in the centre of the nerve. When the eye is reached, the dural sheath becomes continuous with the sclerotic, the pial with the choroid, and the lymph space between them with the perichoroidal space between the lamina fusca and lamina supra-choroidea. At the point where the nerve pierces the fibrous and vascular coats of the eye in this manner, its fibres, which like those of the white matter of the brain and spinal cord generally, have no grey sheath, lose also their medullary, becoming thus non-medullated and passing on as axis cylinders alone. In consequence of this loss, the nerve as a whole becomes narrowed at this point to a kind of "neck," just before it expands into the retina; and it is across this narrowed portion that strands from the sclerotic and choroid pass, forming the *lamina cribrosa*.

After the nerve has passed the level of the pigment layer of the retina, lying immediately internal to the membrane of Bruch, its fibres fall away from an imaginary central axis and spread themselves out in every direction to line the interior of the eye as far as the ora serrata; the central point of divergence of the fibres, a depression surrounded by a raised ring, is termed the *optic disc*. As this part can have none of the retina behind, it is insensible to light, and is called the *blind spot*. The fibres form the innermost of the nervous layers of the retina, the others lying outside between it and the layer of pigment cells on the inside of the membrane of Bruch—the pigment cells representing the retinal portion of the outer wall of the original optic cup. With the exception of the pigmentary layer, the nerve fibres are continuous structurally with all the layers of the retina. On this

account, and also because of the separate origin of the pigment cells from the outer wall of the optic cup, it is sometimes convenient to speak of the "retina proper" as indicating the portion in direct relation with the nervous system, *i.e.*, all the layers except the pigmentary one. But speaking in a more general way, without the intention of drawing a distinction between the nervous and pigmentary layers, the term "retina" may be considered to include both.

The Layers of the Retina (see *Fig. 158*) from within outwards are, shortly, as follows: Lining the inner surface of the nerve fibres is a thin, cuticular sheet, the *internal limiting membrane*; outside this is the layer of *nerve fibres*; then the layer of *ganglionic nerve cells*, with which the fibres are continuous; then a broad, finely granular layer—the *inner molecular*, and external to it the *inner nuclear*; then a narrow *outer molecular*, succeeded by an *outer nuclear*. This latter is bounded on the outer side by a sharply defined *external limiting membrane*, upon which is placed the layer of *rods and cones*. The rods and cones, together with their fibres and nuclei in the outer nuclear layer, may be regarded as the terminal sense cells of the eye, corresponding with the hair cells of the organ of Corti, or with the gustatory cells; and each of them is in continuity, through the intermediate part of the retina, with a nerve filament from the layer of nerve fibres. The rods and cones project externally into the *pigmentary layer*, which is bounded externally by the membrane of Bruch.

In passing to the consideration of the structure of these layers in greater detail, it should be noted that the retina, like other parts of the central nervous system, is made up of neuroglia or supporting tissue in addition to the nervous elements proper. We have thus to consider: (1,) the neuroglial elements; (2,) the nervous elements; (3,) the pigmentary layer; and it is convenient to describe them separately.

1.—*The neuroglial elements of the retina.*

The supporting or neuroglial elements, which, it will be remembered, are derived, not from mesoblast, but from the epithelium of the neural column, are represented by the *radiating fibres of Müller*, which extend from the internal to the external limiting membrane. These fibres are modified, elongated, epithelial cells, which at the ora serrata may be seen to rapidly shorten again, and become continuous with the epithelium of the pars ciliaris retinae. Each fibre possesses a broad expanded foot internally,

the periphery of which fits accurately the space left between the feet of its neighbours; in other words, the expanded basal portions of these fibres, or fibre cells, join together by their margins, to form a continuous sheet—the *internal limiting membrane*. As the fibre is traced outwards through the fibrous and ganglion cell layers, it rapidly narrows, and becomes prismatic in transverse section, delicate processes being given off from its edges to pass amongst the nerve fibres and ganglion cells. As it passes through the inner molecular layer, its edges break up into a close network of fibrils, which contribute to the formation of the layer to a considerable extent. Traced into the inner nuclear layer, the lateral processes alter in character. They are no longer fibrillar, but somewhat plate-like, and form a coarse basket-work arrangement in the meshes of which lie the “nuclei” of the layer. Moreover, in the body of the fibre, as it traverses this layer, is found the nucleus of the original cell from which it has been developed—an oval body with its long axis in that of the fibre itself, *i.e.*, placed radially. In the outer molecular layer the body of the fibre gives off again a close network of fibrils laterally. In the outer nuclear layer the fibre seems to break up entirely to form a basket-work similar to that of the inner nuclear layer, and here the spaces contain the nuclei of the rod and cone fibres. The basket-work ends at the external limiting layer, but its substance appears to be continued a little beyond it in the form of a number of short processes, which fit in between the ends of the rods and cones. The fibres of Müller are said to be composed of neuro-keratin.

2.—*The nervous elements of the retina.*

The nervous elements of the retina may be considered in the following three divisions: (*a*,) The layers of nerve fibres and ganglion cells; (*b*,) The cells of the inner nuclear layer; (*c*,) The layer of rods and cones, and the rod and cone fibres and nuclei of the outer nuclear layer. These different layers are brought into continuity with each other through the medium of the inner and outer molecular layers; but the exact course taken by the connecting fibres or fibrils in these two layers is not yet definitely determined. The processes of the cells of the nuclear and ganglion cell layers appear to break up and join the general network of fibrils, of which the molecular layers are composed; and through this network the impulses travel. But the path may possibly be a more simple one, the processes of

FIG. 154.

S. EYE OF OX (CORNEO-SCLEROTIC JUNCTION), STAINED WITH Picro-CARMINE $\times 20$.

- a.*—Cornea.
- b.*—Sclerotic.
- c.*—Conjunctiva.
- d.*—Iris.
- e.*—Ciliary processes.
- f.*—Ligamentum pectinatum iridis.
- g.*—Pars ciliaris retinae.
- h.*—Blood-vessels.
- k.*—Retina.
- l.*—Ora serrata.

FIG. 155.

S. EYE OF RABBIT, SHOWING ENTRANCE OF OPTIC NERVE, STAINED WITH HÆMATOXYLIN $\times 50$.

- a.*—Retina.
- b.*—Choroid.
- c.*—Sclerotic.
- d.*—Optic nerve.
- d.*¹—Optic disc.
- f.*—Layer of rods and cones.

Fig. 154.

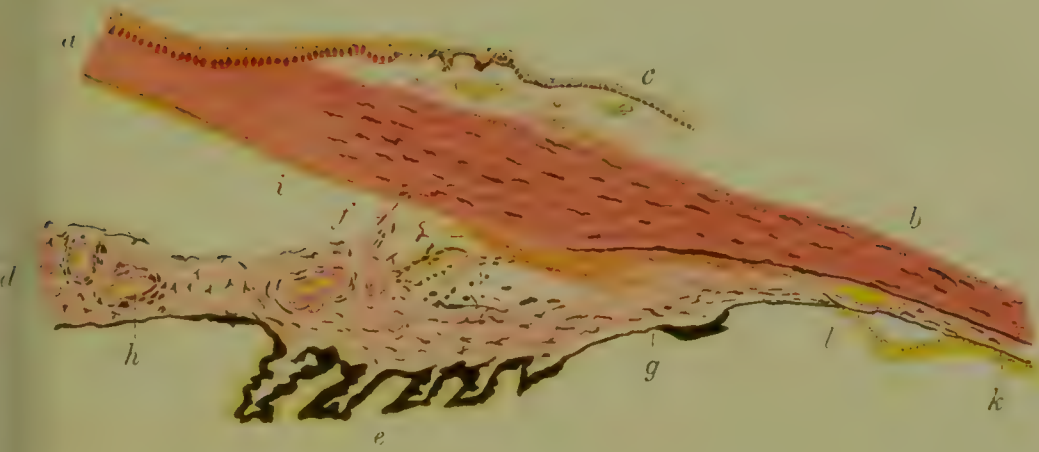


Fig. 155.



the cells in one layer traversing the molecular layer to join those of the next directly.

(a,) *The nerve fibre and ganglion cell layers.*—The non-medulated fibres of the nerve fibre layer wind in and out amongst the basal pieces of the radiating fibres of Müller, and become continuous, in the form of axis cylinder processes, with the cells of the ganglionic layer ; or pass directly into the inner molecular layer in which they appear to break up and become lost. The ganglion cells are somewhat similar in shape to the cells of Purkinje of the cerebellum ; they are large, somewhat globular, possess a large, clear nucleus with a nucleolus, an axis cylinder process, and many peripheral ones. These latter arise from the external pole of the cell in the form of one or two main branches, which pass radially into the internal molecular layer, and in it give off numerous secondary processes which themselves branch again, and ultimately become lost in the general network.

(b,) *The inner nuclear layer.*

Three kind of cells are described :—

(1,) *Bipolar.*—These form the majority, and are cells with a large nucleus, containing a nucleolus, surrounded by little perinuclear protoplasm, placed in the course of a fibre, which passes radially in both directions ; or we may say that the cell is *bipolar*, a peripheral process proceeding towards the outer molecular layer in which it breaks up into a network, a central one terminating in the inner molecular layer in which it becomes lost in a similar manner. These cells, as already stated, form the greater number of the nuclei of this layer.

(2,) *Inner row of unipolar cells.*—These placed near to the inner molecular layer consist, like the bipolar, of a large nucleus with a distinct nucleolus and but little perinuclear protoplasm. They give off one process on their inner side which immediately enters the inner molecular layer, where it breaks up to form a network, and thus becomes lost.

(3,) *Outer row of cells.*—These are rather peculiar in shape, but otherwise, similar to the last. The body of the cell is somewhat flattened in the general plane of the layer. They are placed next to the outer molecular layer, and from their surface numerous branches penetrate it and become lost in the general network.

Both (2,) and (3,) are also said by some observers to possess in

addition an axis cylinder process, which passes through the inner molecular layer and becomes continuous with a nerve fibre.

(c.) *The layer of rods and cones, and the rod and cone fibres and nuclei of the outer nuclear layer.*

The rods are much more numerous than the cones, except at the *macula lutea* in the visual axis of the eye. Here, cones alone are present, and there are other differences in structure which will be referred to when the macula is described. Over the rest of the retina the proportion of rods and cones to each other may be represented somewhat as follows :—

XIIIXIIXIIXIIXIII,

where the cones are represented by x, and the rods by I. A rod consists of two parts ; an outer and an inner segment. *The outer* is cylindrical, transparent, has an appearance of longitudinal striation or fluting, and is liable to break transversely into a number of superimposed discs. In the natural state it is coloured with what is termed *visual purple*, which becomes bleached on exposure to light. The outer segment stains with osmic acid, but not with carmine or hæmatoxylin. *The inner segment* is somewhat spindle-shaped and slightly broader in its middle than the outer segment. One end of the spindle is cut off, viz., that which is in apposition with the outer segment ; the other pointed end projects a little way through the outer limiting membrane into the outer nuclear layer. This membrane is thus pierced with holes to receive the ends of the rods and, as we shall see, it receives the cones too in the same manner. The inner segment itself, exhibits some differentiation into two parts ; that next to the outer segment shows longitudinal striation, while the inner part is finely granular. The whole of the inner, unlike the outer, segment stains with carmine and similar reagents. After piercing with its pointed extremity the external limiting membrane, it is continued as a somewhat delicate filament, which frequently shows varicosities upon it, through the outer nuclear layer. The filament, in some part of its course, bears an oval nucleus, surrounded by a small amount of the substance of which the fibre is composed. The nucleus has the peculiarity of being striped, that is, its chromoplasm, instead of being arranged in the form of a network, assumes the appearance of transverse bars. The filament, or *rod-fibre*, as it is termed, is continued into the outer molecular layer, where it

becomes lost in the general network to which it seems to contribute.

The cones are conical, not cylindrical in shape; they are shorter than the rods, and somewhat broader in their broadest diameter. Like the rods, they consist of two segments, an inner and an outer. The latter is markedly smaller than the corresponding segment of a rod—in fact, the difference in the relative lengths of the rods and cones is mainly due to the shortness of this segment. Its structure appears to be the same as that of the outer rod segments, except that it is not coloured with the visual purple. The inner segment, slightly shorter, and at the same time broader than that of the rods is, in other respects, the same.

The cone, like the rod, pierces the external limiting membrane, and becomes continuous with the cone fibre. But it does not immediately do so. On the inner side of the limiting membrane it undergoes a slight enlargement, which is occupied by a nucleus with an ordinary nucleolus. From the inner pole of this enlargement the cone fibre extends radially to the margin of the external molecular layer, where it expands into a kind of foot, from the inner side of which numerous processes are given off, which become lost in the general network.

Of the *inner* and *outer molecular layer*, very little that is definite can be said at present. The network of which they are composed seems to be contributed to by the processes derived from the fibres of Müller (neuroglial element) and from the cells of the inner nuclear layer and the inner extremities of the rod and cone fibres (nervous element), and this network is imbedded in a homogeneous ground substance.

3.—*The pigmentary layer of the retina.*

The cells of this layer are of a very distinctive character. By their outer surface they are in contact with the membrane of Bruch, from which they may be readily separated. Indeed, under certain conditions, they usually come away with the retina, when it is removed as a whole. Their inner surface, which is always somewhat irregular, is either in contact with the outer ends of the rods and cones, or it is frayed out into filamentous processes which extend between them, sometimes to some distance between their inner segments. The latter condition results if the eye before removal has been exposed to light; the former, if it has been kept in darkness. The two conditions

may be well seen in sections of frog's eyes treated in this way, as shown in *Fig. 159*. The cells are hexagonal in surface view, *i.e.*, when seen from the choroidal aspect; their outlines being indicated by lines, free from pigment, representing the cell walls and intervening cement substance. The pigment is also absent to a large extent from the outer choroidal part of the cell in which the nucleus, itself unpigmented, is found embedded. But the condition of the outer zone varies somewhat with the procession and retraction of the processes on the other (retinal) side of the cell. When these are retracted the pigment is forced back into the outer zone, and the nucleus becomes, partially at least, surrounded by it; the reverse occurring when the processes are extended to their full length.

The protoplasm of these cells is thus capable of amoeboid movement. The pigment, which is in the form of black granules, is termed *fuscine*. When the processes are extended, the layer remains attached to the retina when removed from the choroid; when retracted it adheres to the latter under similar circumstances.

The pigmentary layer is deficient posteriorly where the optic nerve joins the retina. Anteriorly at the ora serrata it passes into the less specialised pigmentary layer covering the ciliary processes.

The larger *blood-vessels* of the retina are found in the layer of nerve fibres, where they are surrounded by perivascular *lymphatics*. The capillaries form a close network both in this layer and around the ganglion cells, but they may extend still further out. In no case do they pierce the outer limiting membrane, and, indeed, they do not usually extend beyond the inner nuclear at the outside.

The **macula lutea**, or **yellow spot**, of about 2 mm. in its longest diameter, is situated a little external to the entrance of the optic nerve, that is, a little to the temporal side of it. It is oval in shape, its long axis being placed transversely. It possesses an outer raised rim of a yellow colour, from which it derives its name, and a central hollow or depression, the *fovea centralis*. In structure, the yellow spot differs markedly from the rest of the retina. The rods begin to grow fewer in number at its margin, and cease altogether in the fovea. The cones, too, are altered in shape. They are considerably elongated, especially their outer segment, which in fact resembles that of a rod, except that it is

thinner. But these are not the only differences. In the raised margin of the macula the increase in thickness is due to a great extent to the layer of ganglion cells, which are here 6 or 8 deep, instead of being, as in the rest of the retina, in a single layer, or, at the most, in two. As the fovea centralis is reached, all the layers thin out and disappear, with the exception of the layer of cones, the outer nuclear layer (consisting here of only cone fibres and their nuclei), and a layer of neuroglia continuous with the molecular layers and internal limiting membrane. In the centre of the fovea, the nuclear and neuroglial layers are so thin that the main thickness of the retina at this point is due to the layer of cones themselves.

If the layer of cone fibres (the outer nuclear layer) be traced from the centre outwards to the marginal rim, a rather curious feature of their disposition may be observed. They no longer run radially to the centre of the eye, or, to put it in another way, vertical to the plane of the retina, as in other parts; they run obliquely, their inner extremity where it meets the outer molecular layer being drawn away from the fovea. As previously stated, the fovea is in the visual axis of the eye, and is the point of acute vision. It will be seen from its construction that light reaches the cones here much more readily than elsewhere. In every other part of the retina before the rods and cones are stimulated, the light must traverse the layers anterior or internal to them; and incidentally it may be mentioned that the nervous impulse then generated, in order to reach the brain, must again traverse these layers, but in an opposite direction, on its way to the optic nerve. But at the fovea the thinness of the layers anterior to the cones renders them especially easy of access.

4. **The lens** is somewhat lozenge shaped. It is perfectly transparent and highly elastic, so that it readily changes its shape under pressure, but returns to its original form when the pressure is removed. It is enclosed in a cuticular capsule, to which, however, it is not structurally adherent, as it can be readily enucleated from it. The capsule is intimately connected on the other hand with the fibres of the suspensory ligament. The lens itself is composed of a number of fibres—hexagonal prisms with serrated edges, which interlock with those of their neighbours. These prisms are developed from the elongation of the posterior cells of the original epithelial sac, from which

FIG. 156.

S. CORNEO-SCLEROTIC JUNCTION OF EYE OF OX, SHOWING DISPOSITION OF BUNDLES OF LIGAMENTUM PECTINATUM IRIDIS, AS SEEN FROM THE ANTERIOR CHAMBER OF THE EYE, STAINED WITH PICRO-CARMINE $\times 40$.

A.—Cornea.

B.—Ligamentum pectinatum iridis ; iris ; and ciliary processes.

a.—Anterior epithelium of cornea.

b.—Fibrous laminæ ,,

c.—Posterior elastic lamina pierced by,

d.—Bundles of ligamentum pectinatum iridis.

f.—Sections of blood-vessels in iris.

g.—Connective tissue of ciliary process.

h.—Epithelium of ciliary process.

e.—Pigment layer of ,,

*d.*¹—Lymph space between bundles of ligamentum pectinatum iridis, lined with squamous epithelium.

FIG. 157.

S. CORNEA OF FROG (CUT IN SURFACE PLANE), STAINED WITH GOLD CHLORIDE $\times 300$.

a.—Corneal corpuscles.

b.—Nerve fibrils.

c.—Connective tissue basis.

Fig. 156.

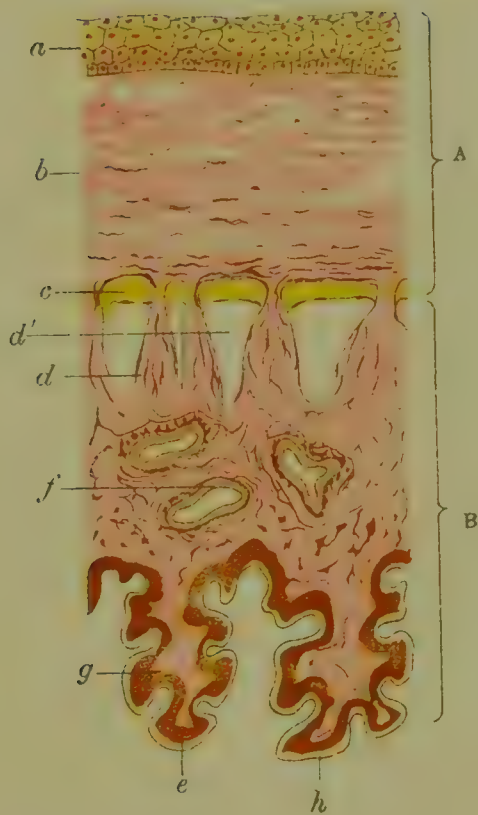
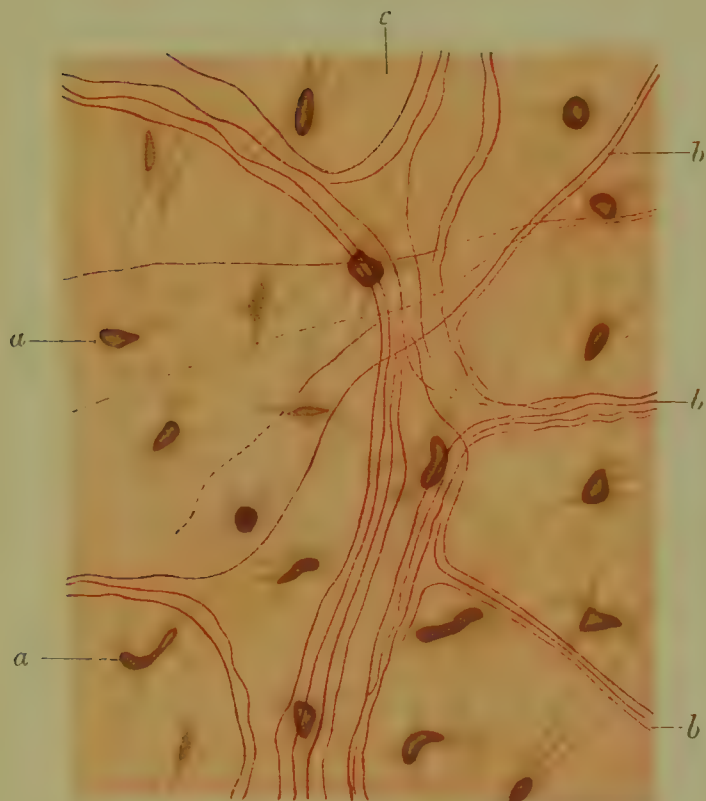


Fig. 157.



the lens is produced. It will be remembered that it owes its origin to an involution of the surface epiblast, which becomes cut off from its source, and enclosed within the orifice of the optic cup, together with a certain amount of mesoblastic tissue. The cells forming the posterior wall of the epithelial sac lengthen so as to obliterate the cavity completely, and continue to do so progressively with the growth of the lens, the nucleus of the cell persisting near its attached end. The anterior cells, *i.e.*, the cells of the anterior wall, remain as they were, cubical in form, throughout life, and constitute what is termed the anterior epithelium of the lens. If the anterior cells be followed round the margin of the lens, the transition between them and the elongated posterior ones may be very easily made out; and this is especially the case in sections of the eye at an early stage of development.

The lens fibres become ultimately arranged in such a way that they form a series of concentric envelopes, comparable with the coats of an onion; and there are other peculiarities in their arrangement, but these need not be referred to here.

The vitreous humour is developed from the mesoblastic tissue which enters with the lens, and which also becomes included in the folded stalk of the optic cup to form the arteria centralis retinae. It is almost structureless, being composed of a very delicate jelly-like matrix, in which are found a few scattered branched cells of the connective tissue type. It is thus regarded as a form of mucous tissue. The whole is invested with a clear, delicate, homogeneous membrane—the *hyaloid membrane*—and within the substance of the vitreous humour a series of more delicate structures of a similar nature are found disposed in a somewhat concentric manner. The investing hyaloid membrane is in contact with the inner limiting layer of the retina, the pars ciliaris retinae, the suspensory ligament, and the lens.

The suspensory ligament.—This is a structure of great importance in connection with accommodation of the eye for near and distant objects. It holds the lens in its place, and by its alternate tension and relaxation (due to alternate relaxation and contraction of the ciliary muscle) it affects the curvature of its anterior surface. It is formed from the mesoblast included in the optic cup. It consists of a sheet of fibrillar tissue, of the nature of white fibrous tissue, surrounding the lens and attached to it in the following manner: The margin of the sheet imme-

diately adjacent to the lens splits into an anterior portion which becomes lost on its anterior surface, and a posterior which follows a similar course behind, both the divisions becoming adherent to the capsule of the lens. The peripheral margin of the sheet also splits in a somewhat similar manner. One-half of it is distributed to the ciliary processes, to which it becomes firmly adherent; its fibres separating from each other to terminate in the drawn-out extremities of the epithelial cells in this region, especially in the furrows between the processes; so that it might be more correct to speak of it as becoming attached to the ciliary enlargement. The other, or posterior half, appears to pass backwards and become lost on the surface of the hyaloid membrane surrounding the vitreous humour.

The zone formed round the lens by the suspensory ligament, is sometimes called the *zonule of Zinn*.

5. **The blood-vessels and lymphatics of the eye** have already been to some extent referred to in describing the structure of the various parts. Their arrangement may be briefly summarised as follows:—

The eye is supplied with **blood** by the *long and short posterior ciliary* arteries, the *anterior ciliary* arteries, and the *arteria centralis retinae*. The blood is returned by the *vena centralis retinae*, the posterior ciliary veins (receiving the blood of the *venae vorticosae*), and the anterior ciliary veins.

The central artery of the retina supplies, as we have seen, the innermost layers of the membrane; the outer layers, including the pigmentary, the layer of rods and cones, and the outer nuclear, are supplied by it and by the capillaries of the chorio-capillary network indirectly.

The short posterior ciliary arteries supply the choroid proper, and their branches run in the main part of the coat to the outside of the chorio-capillary layer in which they terminate.

The long posterior ciliary arteries supply the ciliary processes and iris, which also receive branches from the anterior ciliary arteries. The blood of the long and short posterior ciliary arteries is gathered up from the chorio-capillary layer by the *venae vorticosae*, which terminate in four posterior ciliary veins, which pierce the sclerotic in its equator.

The short and long posterior ciliary arteries both supply the sclerotic.

The anterior ciliary arteries pass with the ocular muscles to

the anterior part of the eye, and, piercing the sclerotic which they supply, contribute to the vascular network of the ciliary processes and iris ; their blood being returned by corresponding veins, the roots of which are in intimate relation to the canal of Schlemm.

The different parts of the eye are supplied as follows :—

The *cornea* is supplied with lymph from the capillaries of the anterior ciliary arteries, which form a network, encroaching slightly upon its margin ; it also receives lymph from the conjunctival capillaries.

The *sclerotic* is supplied by the short and long posterior, and by the anterior ciliary arteries.

The *choroid* is supplied by the short posterior ciliary arteries.

The *ciliary processes and iris* by the long posterior ciliary and anterior ciliary arteries.

The *inner layers of the retina* directly by the *arteria centralis retinae*.

The *outer layers of the retina* indirectly by the *arteria centralis retinae* and chorio-capillary network.

The **lymph spaces** of the cornea and sclerotic are in communication with each other, and with the conjunctival lymphatics. Those of the sclerotic are also connected on the one hand with the *Tenonian cavity* externally, and on the other with the perichoroidal lymph space internally. The *Tenonian cavity* is the space between the sclerotic and *Tenon's capsule*, a loose fibrous tissue investment of the eyeball, which lies outside the sclerotic, and affords sheaths to the tendons of the ocular muscles. Through the medium of the paths of exit of the ciliary veins (posterior) through the sclerotic, the perichoroidal space, and thus the lymphatics of the choroid are in direct communication with Tenon's cavity, which in turn joins the lymph space around the dural sheath of the optic nerve.

The lymphatics of the retina join those of the optic nerve, and these empty themselves into the subarachnoid space between the dural and pial sheaths, and in this way come into communication with the subarachnoid space of the brain (see "Central Nervous System").

The aqueous humour of the anterior chamber seems to be produced by the ciliary processes, perhaps by secretory activity on the part of the epithelial cells of the *pars ciliaris retinae*. It resembles lymph, but contains more water in proportion to solids.

It passes first into the posterior chamber, then into the anterior between the iris and the lens, then through the spaces of Fontana into the canal of Schlemm, and so to the radicles of the anterior ciliary veins.

The posterior part of the globe of the eye, containing the vitreous humour, is filled with a fluid very similar to the aqueous, which percolates through the vitreous as through a sponge, and finds its way through the suspensory ligament into the posterior and anterior chambers. Behind, it is in communication with the lymph spaces of the optic nerve in the region of the optic disc.

THE EYELIDS.

The eyelids are covered externally by a layer of skin, internally by the palpebral layer of the conjunctiva, which becomes continuous with the conjunctiva of the sclerotic at the point of reflection, the fornix. The eyelid is composed largely of a plate of tough fibrous tissue, once termed the "tarsal cartilage," and contains the following structures (see *Fig. 152*): (1,) A series of striped muscular fibres placed circularly immediately beneath the skin, *i.e.*, towards the anterior surface, the fibres of the orbicularis palpebrarum; (2,) posteriorly beneath the conjunctival epithelium a series of some thirty Meibomian glands; (3,) the eyelashes or cilia placed at the junction of the skin and conjunctiva. These hair follicles have sebaceous glands opening into them, and into the ducts of some of these open the glands of Moll, which are of the nature of sweat glands. The cilia separate the strands of the orbicularis palpebrarum nearest the margin of the eyelid from the rest, and these strands have been termed the ciliary part of the muscle, or circular muscle of Riolan.

Examine the following sections :—

(1,) *V. S. Cornea of ox, stained with picro-carmin.* F. (See *Fig. 156*.)

The figure shows especially the relation of the ligamentum pectinatum iridis to the neighbouring structures, but the layers of the cornea are also to some extent shown. Under the low power identify these from before backwards: The anterior epithelial layer, stained both by the carmine and the picric acid; the basement membrane in which it rests; the broad fibrous tissue basis stained pink with the carmine; the posterior elastic lamina stained a bright yellow with the picric acid, bearing on

its posterior aspect a layer of squamous epithelium, which can scarcely be distinguished under this power. With the high power examine the anterior epithelium (*a*), and note that there is here no stratum corneum, the cells at the surface not being completely flattened, and still retaining their nuclei. There is a general resemblance to transitional epithelium, but the number of layers of cells is greater. If the epithelium has by accident become torn at any point, the constituent cells seem readily to separate from each other, and the characteristic "pear-shape" of many of them is well seen. The upper or anterior end of these cells in the middle layers is rounded, their inferior pointed. In the case of the lowest layer of all, the *germinal* layer, the cells are rounded or flattened towards the basement membrane on which they rest. Observe the basement membrane, which stands out as a clear refractile line, and then pass to the connective tissue basis (*b*). Note the succession of superimposed laminæ (seen in section) of white fibrous tissue, of which it is composed, and between them the presence of fusiform cells or cell spaces. The cell spaces are, however, better seen in a specimen stained with nitrate of silver, and the cells in one stained with chloride of gold; at present it will be sufficient merely to note their position between the laminæ.

The posterior elastic lamina (*c*) presents itself as a very well marked, broad, homogeneous, brightly refractile band, stained yellow with the picric acid; and upon its posterior surface the epithelium lining the anterior chamber of the eye is to be looked for. It can always be made out with a little care if it has not been destroyed in preparation, as a thin, often rather granular looking layer, with nuclei occurring in it here and there, stained deeply with the carmine. But in the adult animal it is of the flattened, simple squamous variety, and is not well seen in section. It may be much more clearly demonstrated in the cornea of a young animal such as the kitten, in which the cells are still cubical, and each contains a well marked nucleus, forming a very easily recognisable layer at the back of the elastic lamina.

(2.) *Cornea of frog, stained with gold chloride.* F. (Fig. 157.)

Excise the cornea of a frog, and stain in gold chloride; with a razor or a pair of fine scissors make two or three tangential sections; mount in Farrant. Under the high power note the cells (*a*) in more layers than one (as several laminæ are included in the section), with their branching processes and large nuclei.

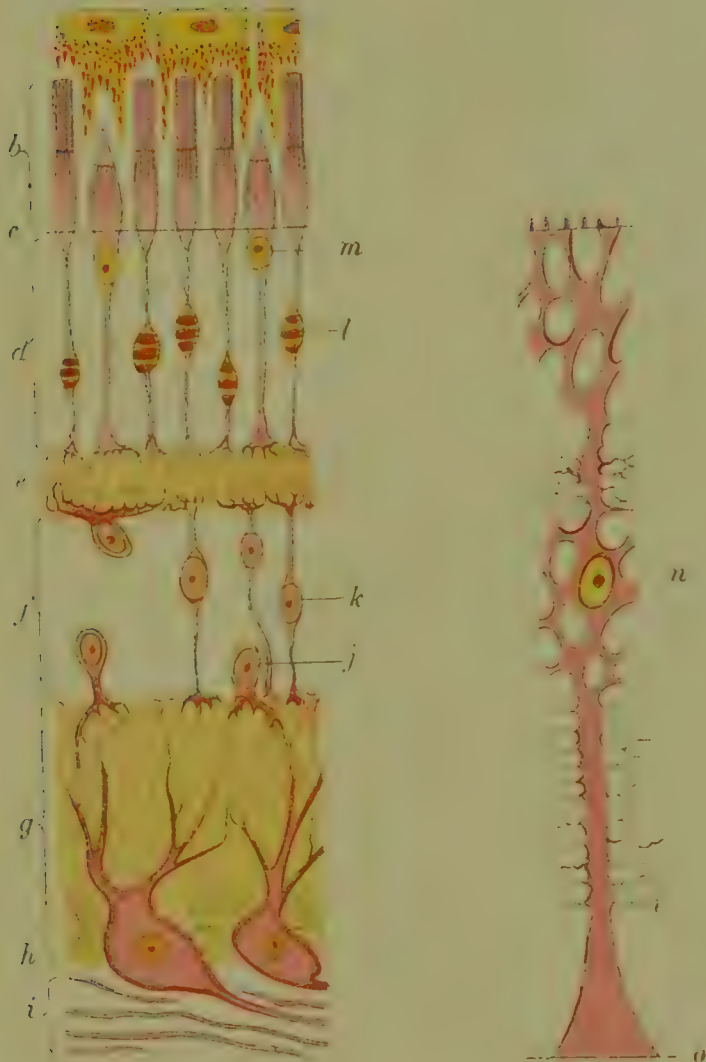
FIG. 158.

DIAGRAMMATIC REPRESENTATION OF STRUCTURE OF RETINA.

- a.*—Layer of pigment cells.
- b.*— „ rods and cones.
- c.*—External limiting membrane.
- d.*— „ nuclear layer.
- e.*— „ molecular layer.
- f.*—Internal nuclear „
- g.*— „ molecular „
- h.*—Ganglion cell „
- i.*—Layer of nerve fibres.
- j.*—Unipolar cells (spongioblasts of some authors).
- k.*—Bipolar cells.
- l.*—Nucleus of rod fibre.
- m.*— „ cone fibre.
- n.*— „ Müller's fibre.
- o.*—Internal limiting membrane.

(*Erratum.*—The letter *a* indicating the pigment cells has been inadvertently omitted from the figure.)

Fig. 158.



Note that the processes anastomose with those of neighbouring cells in the same plane ; by alternating the focus this is very clearly demonstrated, as first one plane of cells and then another is brought into view. Look for nerve fibrils (*b*). The nerves from the sclerotic lose their medullary sheath as they enter the cornea, and form in it plexuses, the strands of which are of varying thickness, being sometimes composed of many fibrils, and sometimes of only one. The single fibrils often show well marked varicosities. From the fact that the sections have been made by hand, and without previous hardening of the tissue, it follows that they frequently present a considerable variation in thickness at different parts when examined under the microscope ; and a thin part, where only one or two layers of cells are included, should be selected. The cells are in this specimen, of course, seen on the flat.

(3.) *V. S. Cornea of rabbit, stained with gold chloride. F.*

Under the high power note again in the body of the cornea the corpuscles, fusiform in section, stained purple with the reagent. Between the fibrous lamella look also for sections of the strands of the *primary nerve plexus*, which are cut obliquely or transversely. Find the basement membrane, and note that it is pierced by fine nerve fibrils, the *rami perforantes*, and study the course taken by these after they enter the epithelial layer. They form first of all a fine network, between the basement membrane and the cells, which is called the *subepithelial plexus*, and from it filaments are given off, which pass as varicose fibrils between the cells themselves, and terminate abruptly anteriorly, without forming any definite epithelial connection. The student will probably require to make more than one of these preparations before a successful result is attained.

(4.) *Cornea of frog, stained with silver nitrate. F.*

Scrape away the anterior epithelium from the cornea of a pithed frog, and rub the denuded surface with solid silver nitrate. Excise the cornea, mount in Farrant, and expose to light.

Under the high power note the clear branching cell spaces, and the intervening matrix stained brown, this specimen being the negative of number 2. Note the character of the spaces and the free anastomosis between them.

(5.) *Meridional section of corneo-sclerotic junction of eye of ox or sheep, stained with picro-carmin. F. (Or in hæmatoxylin and eosin. B.). (Fig. 154.)*

This is one of the most important sections of the eye, and great care should be exercised in mounting it. It is tri-radiate, and shows the junction of the cornea, iris, and sclerotic. The difficulty, especially in mounting from water, is to avoid breaking the ligamentum pectinatum iridis from its attachment to the cornea and sclerotic. It is well to fix the posterior end of the section with the needle, and allow the cornea and iris to float out upon the slide as it is withdrawn from the water. When mounted identify the different parts with the naked eye, and then place the specimen on the stage of the microscope, so that on looking through the tube it will be in the normal position. Under the low power first observe the cornea (*a*) with the four layers already described. Trace the anterior epithelium outwards, and observe the point at which it passes into that of the conjunctiva (*c*). This is a little before the sclerotic is reached, and is marked by the appearance of lightly stained connective tissue, similar to that of the dermis, between the epithelial layer and the tough sclerotic tissue of the eye. Note that the epithelium forms a thinner layer in the conjunctival region than it does over the cornea, and that in the ox and sheep there are frequently pigment granules to be observed in the deeper germinal cells. Now trace the cornea into the sclerotic (*b*), and note that the parallel arrangement of the laminæ ceases, and gives rise to an interlacement instead; moreover, scattered through the sclerotic are to be seen a number of pigment cells, which appear somewhat fusiform in section. Look for the ligamentum pectinatum iridis (*f*). This is very important, and if it is not shown it is advisable to mount a fresh specimen at the earliest opportunity. Note that it appears to be piercing the edge of the posterior elastic lamina of the cornea, which is not reflected over it as frequently stated. Immediately behind and to the outside of the ligament, observe the delicate network formed by the trabeculæ, separating from each other the spaces of Fontana. Look for the ciliary muscle passing backwards from near the corneo-sclerotic junction, to be lost in the anterior part of the choroid. It is recognisable from the radial direction of its bundles, which in a picro-carminic stained specimen have a yellowish pink colour in contrast to the pure pink of the connective tissue. In specimens stained in hæmatoxylin and eosin the muscle is picked out by the eosin.

Pass backwards into the choroid coat, and observe the pig-

ment cells scattered through its substance, and forming a special band separating it from the sclerotic. Where the coat widens out to join the ciliary enlargement two or three large blood-vessels are to be seen in section. On its inner aspect identify the retina (*k*), and trace it forwards to the ora serrata (*l*), where it passes into the pars ciliaris retinæ (*g*). Observe the black line indicating the pigment layer of the retina between it and the choroid ; trace this line forwards also, and note that anterior to the ora serrata it thickens to form the very marked layer of pigment cells covering the ciliary processes (*e*) immediately beneath the pars ciliaris retinæ.

It is important to trace the continuity between these layers in passing from one region to another.

Observe the ciliary processes (*e*), and pass on to the iris (*d*). Note the thick band of pigment at its posterior border continuous with that covering the ciliary processes, and representing, as we have seen, both it and the pars ciliaris retinæ ; the connective tissue basis with its numerous pigment cells ; and the sections of blood-vessels (*h*). Find the anterior surface and trace the iris outwards along this line till it passes into the ligamentum pectinatum iridis (*f*), and so becomes continuous with the connective tissue of the cornea and sclerotic.

Under the high power study the structure of the parts mentioned more minutely, and examine especially the following points. Find the posterior elastic lamina, and trace it outwards till the ligamentum pectinatum iridis is reached. Note particularly the relation of parts here. The ligament (stained pink with the carmine, as it is composed of ordinary white fibrous tissue) pierces the edge of the elastic lamina and becomes lost in the connective tissue of the fibrous tunic of the eye. Observe that outside the point at which the ligament pierces it, the lamina rapidly ends in a point (in section), and also that it is not reflected over the ligament. The flattened epithelium at the back of the lamina, on the other hand, is continued over the ligament and on to the anterior surface of the iris. In these parts it is only recognisable as a thin line with flattened nuclei occurring in it here and there. Examine the spaces of Fontana, which are very well seen in the eye of the ox and sheep, and note the epithelium, continuous with that upon the ligamentum pectinatum, covering the fibrillar trabeculæ bounding them.

If this network of trabeculæ bounding the spaces of Fontana be traced outwards till it merges in the general fibrous basis of the ciliary enlargement, it will be found that the fibrillar part of the trabeculæ is continuous with the white fibres, the epithelial cells investing them with the connective tissue corpuscles, and the spaces of Fontana themselves with the cell spaces. We have, therefore, in this situation a very clear indication of the nature of the communication between the cell spaces of the tissue of the ciliary enlargement and the enlarged lymph space constituted by the anterior chamber of the eye. As far as the connection between the spaces is concerned, this may be very readily and satisfactorily demonstrated by injecting with a hypodermic syringe into the anterior chamber of the eye (through the cornea) red or blue melted gelatine mass. The gelatine mass rapidly passes between the bundles of the pectinate ligament into the spaces of Fontana, and thence into the cell spaces of the tissue beyond. The eye of an ox is very suitable, and it is desirable to have it as fresh as possible. After cooling the eye is incised and hardened, and the corneo-sclerotic junction cut, in the ordinary manner (see "Appendix").

Pass now to the posterior end of the specimen, and study the layers of the sclerotic, choroid, and retina as they are shown in *Fig. 160*. If the retina has broken away in mounting the specimen, the sclerotic and choroid may be examined alone. Especially note the chorio-capillary layer (*d*) forming the inner part of the choroid coat, and the clear line representing the membrane of Bruch between it and the retinal epithelium (*e*). Trace the retina forwards till it passes into the pars ciliaris retinæ, which represents the continuation of the fibres of Müller. Endeavour to make out the transition. Follow the epithelium into the ciliary processes, and observe its characters. Where it covers the ridges the cells are clear, nucleated, and low columnar, or cubical, but in the furrows they are drawn out into long tapering points at their free ends, to which are attached the fibres of the suspensory ligament.

(6.) *Section of corneo-sclerotic junction, tangential to the margin of the cornea, to show the divisions of the ligamentum pectinatum iridis as seen from the anterior chamber; stained with picro-carmine. F. (Fig. 156.)*

This is an exceedingly instructive specimen. Note in it the processes (*d*) of the ligament projecting in a somewhat comb-like manner towards the posterior elastic lamina (*c*), which they pierce to join the connective tissue of the "junction" above. Observe the somewhat triangular intervals (*d'*) between the bundles leading into the spaces of Fontana. Identify the epithelium on the under surface of the elastic lamina, and trace it on

to the subdivisions of the ligament. Observe that it forms a complete lining to the spaces between them.

(7,) *Antero-posterior section of posterior half of eye-ball of rabbit, through the entrance of the optic nerve, stained with hæmatoxylin. B. (Fig. 155.)*

This specimen is especially of value as it shows the way in which the fibres of the optic nerve expand, where they constitute the disc, to form the inner part of the retina, the rods and cones forming the external layer. Observe the optic nerve (*d*) piercing the sclerotic (*c*) and choroid (*b*), to expand into the retina (*a*), of which (*f*) represents the layer of rods and cones. Here, as is so often the case, the retinal pigment epithelium has adhered to the choroid when the retina separated from it. Observe the depression, surrounded by a thickening, forming the optic disc (*d'*).

(8,) *V.S. Retina of ox or sheep, stained with picro-carmin. (Fig. 160.)*

This may already have been seen in the section of the corneo-sclerotic junction, but it is desirable to mount again a piece which has been prepared alone. Under the high power look for the layers as shown in the figure. The retinal epithelium will probably be absent, and the ends of the rods and cones exposed. Note the greater length of the former and their comparatively greater number. Observe the very clear line formed by the external limiting membrane (*f*), upon which the layer is placed. Within or beneath this is to be seen the external nuclear layer (*g*), formed of the rod and cone fibres with their nuclei and the continuation of the fibres of Müller; then the external molecular layer (*h*), followed by the internal nuclear layer (*i*), composed of the bi-polar and other cells with their clearly defined nuclei. In this layer also occur the nuclei of the fibres of Müller. Internal to this look for the broad internal molecular layer (*j*), the layer of ganglion cells (*k*), the fibrous layer (*l*), and the internal limiting membrane (*m*). Placed upon the latter look for the expanded feet of the fibres of Müller, which thin rapidly as they pass upwards (or outwards) through the internal molecular layer. Study also the diagrammatic representation of the layers (Fig. 158) while examining this section.

(9,) *V.S. Retina of frog, stained with borax-carmin. B. (Fig. 159). A, From frog which had been kept in the dark; B, after exposure to sunlight.*

FIG. 159.

V.S. RETINA OF FROG, SHOWING THE EFFECT OF LIGHT AND DARKNESS ON THE PIGMENT CELLS; STAINED WITH BORAX-CARMINE
 × 300.

- A.—Kept in the dark previous to hardening, showing pigment retracted.
 B.—Exposed to light; showing pigment extending down between the rods.

- a.*—Pigment cell layer.
b.—Layer of rods and cones.
c.—External limiting membrane.
d.—Outer nuclear layer
e.— „ molecular layer.
f.—Inner nuclear „
g.— „ molecular „
h.—Ganglion cell layer.
i.—Internal limiting membrane.

FIG. 160.

V.S. SCLEROTIC, CHOROID, AND RETINA OF OX, STAINED WITH PICRO-CARMINE × 200.

- A.—Sclerotic (inner part of).
 B.—Choroid.
 C.—Retina.

- a.*—Fibrous tissue of sclerotic.
b.—Lamina suprachoroidea.
c.—Body of choroid, containing large vessels.
d.—Chorio-capillary coat.
e.—Pigment layer of retina.
f.—External limiting layer.
g.— „ nuclear „
h.— „ molecular layer.
i.—Internal nuclear „
j.— „ molecular „
k.—Ganglion cell layer.
l.—Layer of nerve fibres.
m.—Internal limiting membrane.

Fig 159

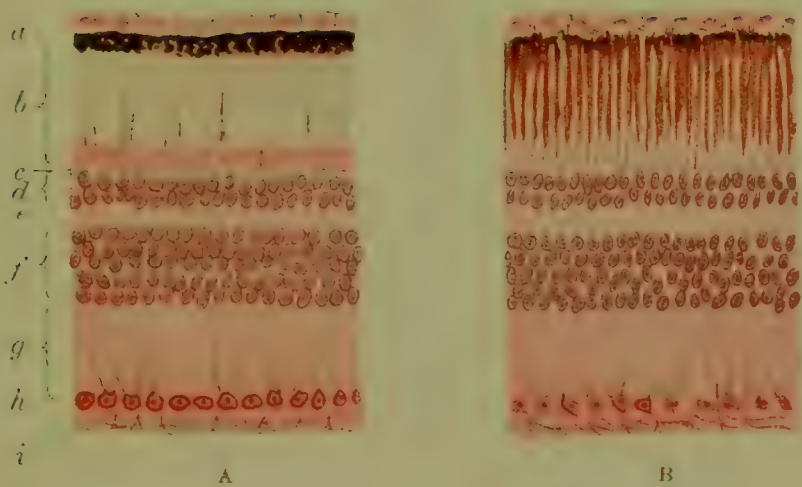
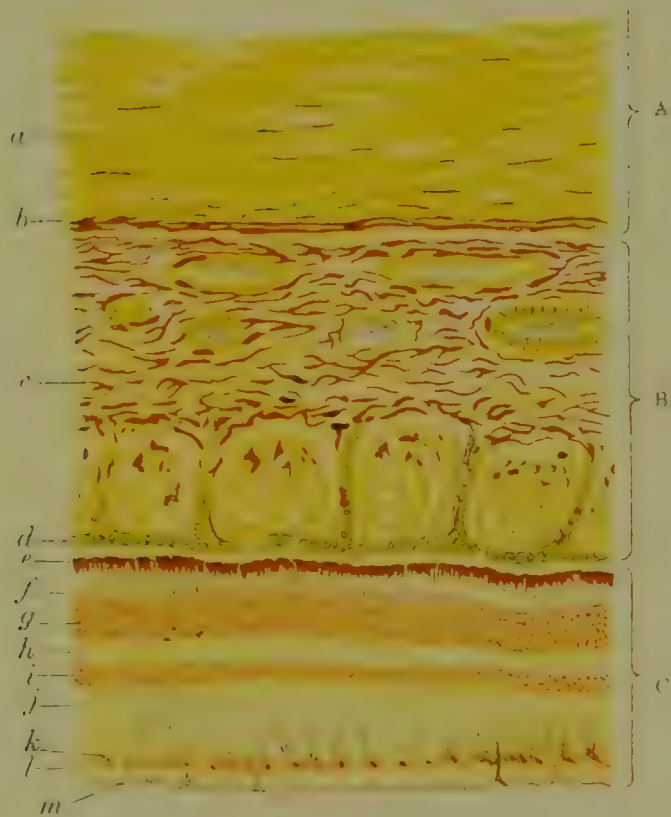


Fig. 160.



Examine this specimen for a general view of all the parts of the retina as already described, and especially for the pigmentary layer. Observe that in (A) the pigment is retracted, and forms a comparatively narrow band ; in (B) it is extended between the rods and cones in the form of delicate filamentous processes of protoplasm laden with pigment. Refer also to the pigment layer in *Fig. 158*, in which the character of the individual cells is more distinctly seen.

(10,) *Rods and cones of frog's retina, isolated, stained with osmic acid. F.*

Bisect a frog's eye in its equator, and place the posterior half in osmic acid (1 per cent. solution) for twelve hours, and tease in Farrant's solution.

Note the numerous rods and cones scattered over the field. Observe the outer and inner segments of the rods, the former of which is blackened with the reagent, and may show a tendency to split transversely. Look also for other parts of the retina—the nuclei of the nuclear layers, a few ganglion cells from the nerve cell layer, and pigmented epithelium. Note that the half of the cell containing the nucleus is unpigmented.

(11,) *Section of head of chick showing antero-posterior section of developing lens, stained with borax-carmin. B.*

Under the high power study the lens, the essential structure of which is well seen during its development. Note the anterior epithelium consisting of a single layer of cubical nucleated cells. Trace them backwards, and note the transition between them and those of the posterior wall, which are elongated so as to entirely fill the cavity of the original epithelial sac, and come into contact with the cells of the anterior layer.

(12,) *Isolated lens fibres of fish, unstained. F.*

Tease a small fragment of the boiled lens of a cod-fish, and mount in Farrant. Under the high power note the lens fibres, clear, colourless, and transparent, with a denticulate margin. The serrated edges of adjacent fibres interlock. The fibres are modified epithelial cells, representing the further development of the posterior layer of the embryonic lens.

(13,) *T. S. Optic nerve of ox, stained with hæmatoxylin. B.*

Under the low power note the general fibrous sheath sending in septa, dividing the nerve into somewhat irregularly polygonal areas of nerve fibres. Note that there is no lamellar perineurium, and the nerve bundles are not cylindrical as in an ordinary cerebro-spinal nerve.

(14,) *V. T. S. Eyelid of child, stained with hæmatoxylin. B. (Fig. 152.)*

Examine especially under the low power the general arrangement of parts as shown in the figure. Note that the so-called tarsal cartilage in which lie the Meibomian glands (*d*) is not in reality cartilaginous, but consists of a plate of condensed connective tissue. Observe the opening of the Meibomian glands at the margin of the eyelid (*c*). Note that the cilium or eyelash (*a*) divides the orbicularis muscle (*b*) into an outer and an inner portion, of which the latter (*e*) has received the special name of the *circular muscle of Riolan*, or the *ciliary part of the orbicularis palpebrarum*. Between it and the cilium note the modified sweat glands (*f*), which are known as the *glands of Moll*. Under the high power study the stratified squamous epithelium investing the eyelid on both external and conjunctival surfaces, the large hair follicles of the cilia, the transverse sections of the muscular fibres of the orbicularis palpebrarum, the glands of Moll, the Meibomian glands, etc.

THE EAR.

The internal ear commences as an *otic vesicle*, budded off from the epiblast. At first connected with the surface epithelium, it presently becomes entirely separated from it, and sinks further into the tissue beneath. The sac, lined by a single layer of cubical epithelial cells, is ultimately met by the divisions of the auditory nerve projected from the brain, and at the points of contact the lining epithelium undergoes special development and becomes directly continuous with the nerve fibres. Thus the ear differs from the eye in its development in several points. In the first place, the otic vesicle is derived from the epiblast directly, and not through the medium of the neural column. It is not, therefore, like the optic cup, to be regarded as an outlying part of the brain; and as we shall see when its structure is considered minutely, it resembles much more nearly the organs of taste and smell. In these we have to deal, as in the ear, with specially modified cells of epiblastic origin, resting upon a dermis of connective tissue, through which nerve fibres pass to connect the proximal ends of the cells with the central nervous system. In the second place the epithelial sac does not undergo invagination, as the optic vesicle does to form a cup, thus obliterating its

cavity ; but the latter remains throughout life, and is occupied by a fluid termed *endolymph*. Thirdly, a much smaller portion of the vesicle comes into relation with the nervous system. In the case of the optic cup, the whole of the anterior wall behind the ora serrata, with the exception of the blind spot, is sensitive to light. In the otic vesicle only the special areas, where the branches of the auditory nerve join the epithelium of the sac, are related to special sense.

Though the plan of construction remains the same throughout, the otic vesicle in the course of its development undergoes changes by which it becomes apparently a very complicated structure. Its general contour changes, so that it no longer forms a simple sac, but a series of canals and cavities which, however, still remain in connection with each other. But changes also take place in the mesoblast around the otic vesicle, as they do around the optic. Here the mesoblastic tissue immediately surrounding the epithelial sac affords it a fibrous investment which follows its contour closely, and with it constitutes what is known as the *membranous labyrinth*. Further out, the mesoblast develops into bone, the inner condensed shell of which, when dissected out from the rest, is found to correspond roughly in form with the membranous sac it encloses, and is termed the *osseous labyrinth*. It corresponds, we have said, roughly with it ; that is to say, it does not follow its contour exactly, as for instance, where one division of the osseous labyrinth—the vestibule—encloses two, the utricle and saccule, of the membranous ; and, moreover, through the greater part of their extent the two labyrinths are separated from each other by a very well marked lymph space, containing a fluid termed *perilymph*. The space is not complete, but is broken in various places ; sometimes by the membranous and bony labyrinths touching each other at some particular point, when the fibrous layer of the one fuses with the periosteum of the other ; sometimes by a bridge of connective tissue extending between the two ; sometimes by a branch of the auditory nerve on its way to its termination in the epithelial lining. The perilymph space is lined by ordinary lymphatic epithelioid cells with sinuous outlines, and is continuous with the lymph spaces in the connective tissue which bounds it.

The Membranous labyrinth consists of :—

- (1,) The Utricle and Semicircular Canals.
- (2,) The Saccule.

(3.) The Canalis Cochlearis.

The *utricle* (Fig. S, *u*) is a somewhat oval sac with which the *semicircular canals* (*s. c*) communicate by either end. Each semicircular canal possesses an enlargement, termed an *ampulla* (*a*), at one of its openings into the utricle. The utricle communicates somewhat indirectly by means of the *ductus endolymphaticus* (*d. e*), with the smaller and rounder *sacculæ* (*s*). From the latter a narrow tube, the *canalis reuniens* (*c. r*), leads to the *canalis cochlearis* (*c'*), which terminates blindly at the apex of the spiral into which it is twisted.

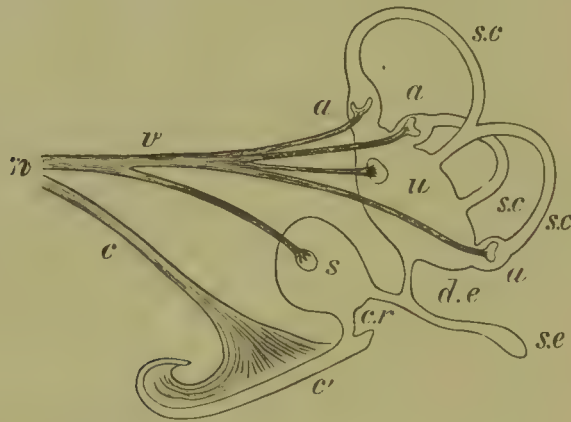


Fig. S.—Diagrammatic representation of membranous labyrinth, and branches of auditory nerve: *n*.—Auditory nerve; *v*.—Vestibular branch; *c*.—Cochlear branch; *s. c*.—Semicircular canals; *a*.—Ampullæ; *u*.—Utricle; *s*.—Saccule; *d. e*.—Ductus endolymphaticus; *s. e*.—Saccus endolymphaticus; *c'*.—Cochlea; *c. r*.—Canalis reuniens.

The relation of the membranous labyrinth to the walls of the space in which it is enclosed, as already described, applies more particularly to the *vestibular* part (utricle and saccule) with the semicircular canals. The *cochlear* portion of the membrane—the most important part of the ear in connection with the analysis of sound—differs markedly in its disposition, its relation to the surrounding bone, and its nerve supply, from the rest. In the first place, it is coiled as a spiral in two and a half whorls round a central bony stem, or *modiolus* (Fig. T, B g), with the result that the osseous labyrinth here has a strong resemblance to a snail's shell, and has, consequently, been called the *cochlea*; the contained portion of the membranous labyrinth being termed the *canalis cochlearis*. The relation of the canal, or more properly speaking, membranous tube, to the bony space which contains it, is somewhat as follows. In a vertical section of the structure, the cavity of the bony canal (cut five times transversely), is seen

in section surrounding the central modiolus, or stem of the shell (*g*). From this aspect it is somewhat round as a whole, but is partially divided into two secondary canals by a bony plate, the *osseous spiral lamina* (*f*), projecting horizontally from the central modiolus towards the outer wall. The *canalis cochlearis*, triangular in shape in transverse section, is so placed in the bony canal of the cochlea that the narrow base of the triangle is in complete contact with the outer wall, while the opposite angle is connected with the osseous spiral lamina.



Fig. T.—A. Bony-labyrinth dissected out from rest of temporal bone ; B. Bony cochlea in vertical section.

a.—Semicircular canals ; *b*.—Ampullæ ; *c*.—Vestibule ; *d*.—Macula and crista ; *e*.—Cochlea ; *f*.—Osseous spiral lamina ; *g*.—Modiolus.

This relation of parts is maintained throughout the two and a half turns of the spiral, and the *canalis cochlearis* has thus a very definite and constant relation to the perilymph space, which the rest of the membranous labyrinth lacks.

On its outside, the side in contact with the outer wall of the cochlea, the space is entirely absent, the membranous and osseous labyrinths being intimately adherent. Above and below, on the other hand, it is bounded by the lymph space throughout its whole extent. The lymph space is necessarily divided into two by the *canalis cochlearis* itself and the osseous spiral lamina, which projects from the modiolus to meet it ; and the two spaces thus produced have received different names. The one above the *canalis* is found to terminate at the base of the cochlea in the vestibular perilymph space (*i.e.*, the space surrounding the saccule and utricle), and is called the *scala vestibuli*. The one below the *canalis* terminates at the base in the *fenestra rotunda* (Fig. U, *tr*), the membrane of which completes the wall of the middle ear or tympanum at this point ; it is hence termed the *scala tympani*.

At the apex of the cochlea the arrangement of the two *scalæ*

is simple, though at first it is not easy to form a mental picture of it. The canalis cochlearis of the membranous labyrinth terminates at the end of the spiral blindly, and at this point the ossaceous spiral lamina, its purpose gone, ends in the form of a

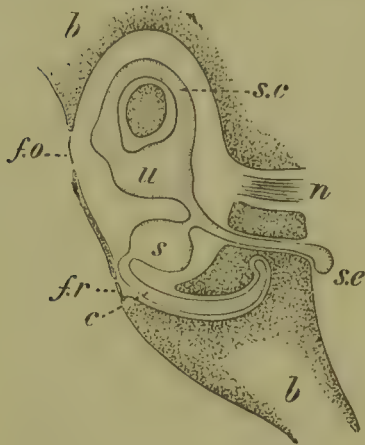


Fig. U.—Diagrammatic representation of internal ear: *b.*—Bone; *s.c.*—Semi-circular canal; *u.*—Utricle; *s.*—Saccule; *c.*—Cochlea; *f.o.*—Foramen ovale; *f.r.*—Foramen rotunda; *n.*—Auditory nerve; *s.e.*—Saccus endolymphaticus

bular (*Fig. S, v* and *c*). The latter divides again to form five smaller strands, which are distributed as follows: One to each of the ampullæ of the semi-circular canals, one to the utricle, and one to the saccule. The small oval swelling, on the inner surface of the utricular wall in which the nerve ends, is termed the *macula acustica*, and a similar spot is found in the saccule. The localised epithelial modifications in which the nerves to the ampullæ of the semicircular canals terminate, are termed *cristæ acusticæ*. The distribution of the nerve, therefore, in all these situations is limited to one part of the epithelial lining alone. In the cochlea, however, the nerve is supplied to the modified epithelium along a portion of one side of the entire length of the canalis cochlearis, from its commencement at the base to its termination at the apex, this spiral line of modified epithelium being known as the *organ of Corti*.

THE STRUCTURE OF THE MEMBRANOUS LABYRINTH.

The Maculæ and Cristæ acusticæ.—The structure of the walls of the utricle, saccule, and semicircular canals, has already been to some extent indicated. They are lined by a layer of polygo-

hook, the *hamulus*; with the result that the two scalæ, the partition between them removed, form one canal at this point. Thus, if the perilymph be followed from the vestibule, it is found to pass from the base to the apex of the cochlea along the scala vestibuli, and from the apex to the base (where it impinges against the membrane of the fenestra rotunda) by the scala tympani. (See *Fig. U.*)

The cochlear portion of the membranous labyrinth also differs from the rest in the manner in which the nerve is distributed in its wall. The auditory nerve divides into two main branches, the *cochlear* and the *vesti-*

nal, flattened, nucleated cells, resting upon a thin hyaline surface condensation of the connective tissue beneath, termed the "tunica propria." Outside this is the general vascular fibrous investment, surrounded by the space containing perilymph; a space, lined by the usual lymphatic epithelioid cells with sinuous outline, and broken in different places by bridles of connective tissue, uniting the membranous labyrinth with the periosteum of the bone. The perilymph space is in communication with the lymph spaces of the connective tissue on either side of it, and on the other hand, with the lymphatic space beneath the dural investment of the auditory nerve. The maculæ and cristæ acusticæ, in which the vestibular branches of the nerve terminate, require further description.

The *macula acustica* of the utricle (or of the saccule) forms an oval thickening of the vestibular wall, a vertical section of which appears to show the following structure. Both the epithelial layer and the dermis of connective tissue by which it is surrounded contribute to form the thickening. The dermis constitutes a well marked cushion of connective tissue, the superficial layer of which immediately beneath the epithelium is, more or less, homogeneous, non-nucleated, and non-vascular. Deeper down, the ordinary characters of connective tissue assert themselves. The epithelium covering the cushion consists of more than one layer of cells. Those that are superficial are cylindrical in form, and only extend a third, or a little more, of the distance between the surface of the epithelium and the hyaline layer of the dermis, where they terminate in a rounded free end, in which is placed a rounded well marked nucleus. From the other end of each cell there projects into the cavity of the sac a bayonet-like process, apparently composed of a number of "hairs" stuck together, as those of a paint-brush are when it is wetted. It may be mentioned, in passing, that these processes are much more strongly marked than those of the hair cells of the organ of Corti; but less so than those of the hair cells of the cristæ acusticæ.

Between these *hair cells* and the dermis are a number of layers of nuclei which belong to the second variety of cell found in the maculæ. These are the *supporting*, or rod-like cells, extending from the surface of the epithelium (where they probably join a cuticular membrane pierced by, and so supporting the inner ends of the hair cells) to the dermic layer beneath. These rods or fibres contain each at some part of its course (but always

beneath or to the outside of the hair cells) an oval nucleus, which varies greatly in its position in adjacent fibres, so that an appearance of several layers is produced in vertical section.

At the margin of the swelling, the complicated epithelium covering the dermic cushion, becomes suddenly resolved into a layer of tall columnar, nucleated cells, which in turn become polygonal and flattened as the general level of the sac wall is reached.

The nerve fibres of the branch of the vestibular nerve to the macula, spread out fan-wise as they pass through the dermic cushion. They retain their sheaths, grey and medullary, here, but lose both as they enter the epithelial layer. They then pass as naked branching axis cylinders between the supporting cells, and each ends in a tuft or nest of nerve fibrils, which receives the rounded end of one of the hair cells, much as the end of an acorn is received by its cup.

Above the hairs of the hair cells, and apparently maintained in its position by contact with their extremities, is a structureless membrane of a somewhat mucinous nature, containing rhombic or octohedral crystals of calcium carbonate. It possesses interesting analogies to similar structures in the membranous labyrinth of the ears of less highly developed species, and is sometimes termed the *otolith membrane*, sometimes the *otolith*.

The *cristæ acusticæ* of the ampullæ of the semicircular canals are similar in structure to the maculæ, except that the shape of the thickening is different, being of the nature of a ridge or crest, the otolith membrane is absent, and the processes of the hair cells are more marked.

The Cochlea and organ of Corti.—The osseous spiral lamina, as already stated, projects outwards from the modiolus as a spiral shelf, which extends a certain distance across the bony canal of the cochlea, and in conjunction with the membranous tube, divides it into two divisions, an upper, the vestibular, and a lower, the tympanic. The edge of the spiral lamina is thin, but the connective tissue covering it, or periosteum, is developed in a special manner upon its upper surface to form a thickening, which is termed the *limbus laminæ spiralis*. This is hollowed out on its outer aspect so as to present two lips externally, the upper of which is termed the *labium vestibulare*, and the lower, the *labium tympanicum*. The hollow between the two is termed the *sulcus spiralis*. The connective tissue of the thicker or vesti-

bular portion of the limbus is peculiar in character. It consists of a strong, apparently homogeneous matrix, from which cells appear to be absent in the more superficial part, which is, moreover, split at the edge of the lip radially into a series of auditory *teeth*, which appear somewhat like the keys of a piano when looked at from above. From the upper surface of the vestibular portion of the limbus, or *crista spiralis*, as it is sometimes termed, projects the *membrana tectoria*, which stretches outwards over the *organ of Corti* itself.

From the upper surface of the limbus, the superior* wall of the canalis cochlearis, or *membrane of Reissner*, passes upwards and outwards to meet the periosteum of the bony canal with which its connective tissue basis becomes continuous; while the epithelium of its under surface is reflected upon the inner surface of the periosteum, as the external side or base of the cochlear triangle. The membrane of Reissner is composed of a thin median layer of delicately fibrillated or homogeneous tissue, continuous on the one hand with the connective tissue of the limbus, and on the other, as already stated, with the periosteum of the outer wall of the bony canal. On the vestibular, or upper side, the membranous sheet is covered with ordinary lymphatic epithelioid cells, continuous with those of the rest of the scala vestibuli; on the other, with a layer of flattened epithelial cells with a polygonal outline, resembling those lining the general cavity of the vestibular part of the membranous labyrinth, and continuous with the cells of the organ of Corti.

These flattened polygonal cells when followed to the limbus are found reflected upon its surface, with the exception of the auditory teeth, between which they sink; traced downwards they pass into a layer of cubical or columnar cells lining the sulcus spiralis, and these again into the organ of Corti. It has been stated that the epithelium of the inferior surface of the membrane of Reissner becomes reflected upon the periosteum of the external wall of the cochlea, and here the periosteum is peculiar and deserves special attention. It is considerably thickened, and its thickness increases as it is traced downwards till a point is reached where the epithelium again leaves it to pass inwards upon

*The parts are here described in their relation to each other when the cochlea is removed from the body and placed vertically, with its apex above, and its base below. This is not its natural position, but the most convenient in which to describe or examine it.

FIG. 161.

SECTION OF TEMPORAL BONE OF GUINEA-PIG, SHOWING TWO TURNS OF THE COCHLEA AND THE EUSTACHIAN TUBE IN TRANSVERSE SECTION, STAINED WITH PICO-CARMINE $\times 20$.

- a.*—Organ of Corti.
- b.*—Scala vestibuli.
- c.*— „ tympani.
- d.*— „ media.
- e.*—Membrane of Reissner.
- f.*—Spiral ganglion.
- g.*—Ligamentum spirale.
- h.*—Epithelium (ciliated) of Eustachian tube.
- i.*—Cartilage of Eustachian tube.
- j.*—Bone.

FIG. 162.

V.S. COCHLEA OF GUINEA-PIG, STAINED WITH PICO-CARMINE $\times 20$.

- a.*—Auditory nerve (cochlear branch) in modiolus.
- b.*—Ganglion spirale.
- c.*—Organ of Corti.
- V.—Scala vestibuli.
- M.— „ media.
- T.— „ tympani.

Fig. 161.

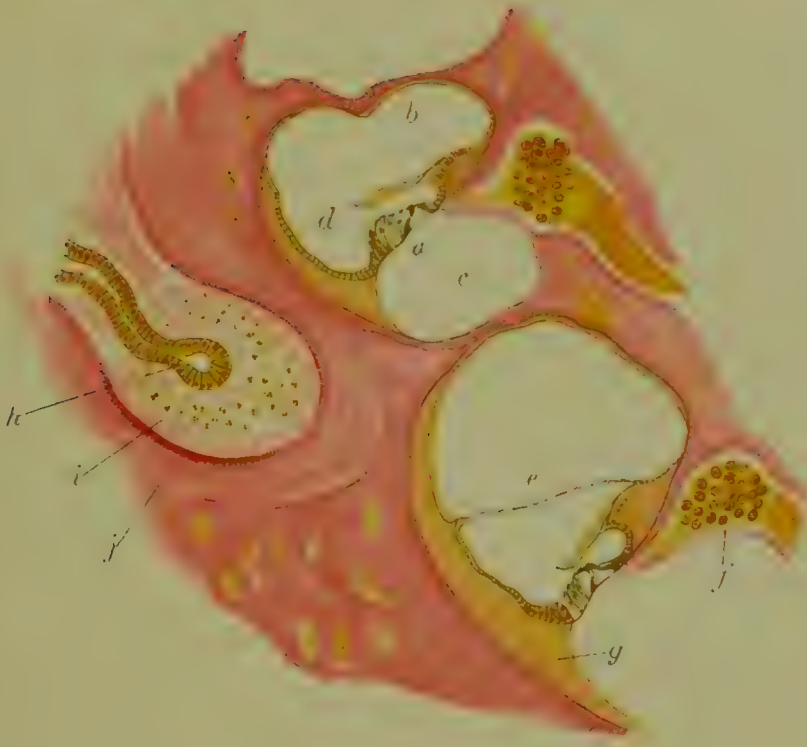
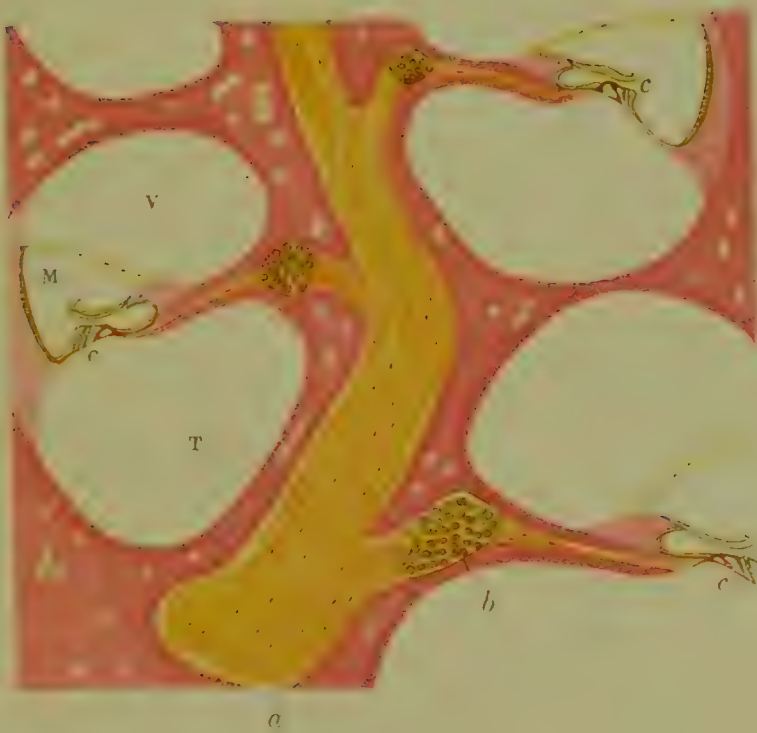


Fig. 162.



the membranous spiral lamina. Below this point it gradually decreases in thickness again as it lines the outer wall of the scala tympani. At the line where the epithelium is carried inwards the periosteum is thus at its thickest, and its fibres converge to a point (in section) into which the tissue is seemingly drawn to meet the basilar membrane. This projecting line (or point in section) is termed the *spiral ligament*.

If the epithelium be traced from the inferior (or inner) surface of the membrane of Reissner on to the outer wall of the cochlea, where it lines this thickened periosteum, it will be found to alter very much in character. The cells become tall, columnar, and granular, and have this peculiarity, that capillary vessels, from the connective tissue upon which they are placed, penetrate between them. The line formed by these vessels, as seen from the *canalis cochlearis*, has received the name of the *stria vascularis*.

The basilar membrane extends from the spiral ligament to the tympanic lip of the limbus, and represents the fibrous investment of this part of the membranous labyrinth. Its structure, however, shows considerable modification. In its inner part, between the tympanic lip and the organ of Corti, it consists of a solid sheet of tissue, which is, however, radially striated. Externally to this it breaks up into radiating fibres embedded in a homogeneous matrix. Its tympanic or inferior surface is covered by a layer (or, it is said, more than one) of lymphatic fusiform cells, continuous with those lining the scala tympani generally. Between these cells and the membrane, and beneath the tunnel of Corti, runs a spiral vessel, the *vas spirale*. The epithelium covering the upper or cochlear surface is continued, on the one hand, into that lining the sulcus, and, on the other, it passes into the cells of the epithelium covering the stria vascularis. Its middle portion is specially modified to receive auditory impressions, and this part is named the organ of Corti.

In a vertical section of the cochlea, if the cubical epithelium lining the sulcus spiralis (*Fig. 164, b*) be traced outwards, the cells may be seen to become rapidly columnar in shape, and thus to attain a considerable height before the membrana basilaris is reached. The last (*i.e.*, the outermost) of these columnar cells is specially modified, and is known as the *inner hair cell*. It is not unlike one of the cylinder or hair cells of the macula or crista, and, like it, possesses a conical or rounded lower extremity, containing a well marked nucleus. This extremity of the cell only reaches

half the depth of the epithelial layer as a whole, which beneath this level appears to consist of a series of *nuclei*, similar to those of the macula and crista. They are the nuclei of narrow, supporting cells, the lower extremities of which rest upon the connective tissue covering the osseous spiral lamina, while the upper extend to the level of the surface of the epithelium, where they end in a cuticular formation. The free surface of the cylinder cell is marked by a hyaline border, not unlike that of ciliated epithelium, and from the surface of this plate project ten to fifteen short stiff hairs or rods, which are arranged in a somewhat peculiar manner.

They do not arise from the general surface of the end of the cell, but in the line of a curve extending across it, in such a way that the concavity is directed towards the sulcus. As we shall see, the hairs of the outer hair cells are placed upon them in a somewhat similar manner, but in the latter case the curve is much more acute, so much so as to be horse-shoe shaped.

As the edge of the osseous spiral lamina is reached, the *rods* or *pillars of Corti* come into view. These (in vertical section) are two in number, and have a characteristic shape. Each possesses a *head* with a process projecting from it externally, a *body* or *limb*, and an expanded *foot*. They are so arranged in relation to each other that the head of the outer rests in a corresponding concavity in that of the inner, while the limbs of both slope apart, the one to the inside and the other to the outside, so that there is a considerable distance between their feet, which are cemented to the basilar membrane. The space, somewhat triangular in section, bounded above by the limbs of Corti's rods, meeting like the rafters of a roof, and below by the basilar membrane, constitutes the *tunnel of Corti*, which winds spirally with the other structures of the membranous spiral lamina round the central stem or modiolus of the cochlea. The rods are probably cuticular in nature, and their origin is indicated by the presence of a nucleus surrounded with undifferentiated protoplasm on the tunnel side of the foot of each rod. The protoplasm may even extend as a thin layer upwards along the limbs of the pillars. In the fresh state these are apparently homogeneous, or there may be some indication of longitudinal striation, which, however, becomes much more pronounced under the influence of reagents. Their cuticular nature is shown, too, by the fact that the processes

projecting externally from their heads are continuous with the *membrana reticularis*, itself of obvious cuticular origin, which receives and supports the ends of the outer hair cells.

The rods of Corti form a spiral tunnel, as already stated, but a tunnel whose sides are incomplete. Though the heads and feet of adjacent rods in the same row are in contact along the length of the spiral, the limbs which are more slender necessarily do not touch each other, and it is through these clefts between adjacent rods that the branches of the cochlear nerve reach the outer hair cells. Passing still further outwards four rows of other hair cells are to be seen in the human subject, though this number does not necessarily hold good in every part of the spiral. In many animals the usual number is three. Beyond the hair cells a series of very tall columnar cells (*Hensen's*) (*m*) form the highest part of the organ of Corti, but when traced outwards they suddenly decrease in length, and are continued along the rest of the basilar membrane as the *cells of Claudius* (*n*), which are cubical in form, and join with those covering the *stria vascularis* upon the spiral ligament.

The *outer hair cells* have a still more complicated structure than the inner. Each may be regarded as consisting of two parts: (1,) the hair cell proper, or Corti's cell; (2,) the supporting, or Deiters' cell. The two are in close contact with each other at one part of their course, and, indeed, are by some regarded as being in structural continuity. The hair cell proper resembles the cylinder cell of the macula or the inner hair cell, and, like it, ends about the middle or upper third of the layer in a rounded or conical extremity, containing a well marked nucleus. The upper end of the cell possesses a hyaline border, and immediately beneath this is a somewhat nuclear looking structure, *Hensen's body*. The upper end of the cell is surrounded by one of the *rings* of the cuticular *membrana reticularis*, and from it project some ten or twelve hair-like processes in the form of a horse-shoe, the concavity of which is directed inwards towards the *sulcus*.

The *cell of Deiters* lies to the outer side of its twin cell of Corti. It is a comparatively long and narrow cell, and extends from the under surface of the *phalanx* of the cuticular membrane immediately to the outside of the ring grasping the end of the hair cell to the *membrana basilaris* beneath. This it meets at an acute angle, as it slopes outwards as it descends. About its

middle, or a little higher, the lower end of the hair cell to which it belongs, abuts against it, and is probably fused or cemented to it; towards its lower end it increases somewhat in thickness to accommodate a nucleus. Each supporting cell is strengthened by a cuticular band, which is fused to its inner side where it joins the *membrana basilaris*, but higher up can be traced into the central axis of the cell in which it runs to meet the *membrana reticularis*. These cells of Deiters seem to correspond with the rod cells of the macula and crista, but possess a more complicated structure.

The *membrana reticularis* is probably cuticular in nature. It extends from the phalangeal processes of the rods of Corti over the area occupied by the outer hair cells, and terminates at the edge of Hensen's cells. It consists of *rings* or *annulæ*, separated from each other by flat bars or *phalanges*. The rings receive the ends of the outer hair cells, while the cells of Deiters are

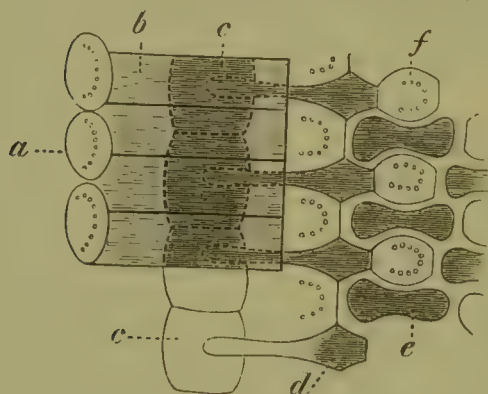


Fig. V.—Surface view of part of organ of Corti (after Retzius): *a*, Inner hair cell; *b*, Heads of inner rods; *c*, Heads of outer rods, with *d*, Phalangeal processes; *e*, Phalanges, *f*, Second row of outer hair cells.

attached by their upper extremities to the phalanges. But the disposition of these parts cannot be adequately studied in a vertical section of the organ of Corti. To see the relative position of the processes from the heads of the rods of Corti, of the rings, and of the phalanges, a surface view of these structures from the scala media is required. When viewed thus the phalangeal processes of the rods

give somewhat the appearance of the keys of a piano, as may be seen in the accompanying diagram (Fig. V).

The *membrana tectoria* is composed of a soft elastic substance, and exhibits a fine radial fibrillation. It varies somewhat in shape, but may be said generally to be a thick membrane, which, however, becomes thin at its point of attachment and free margin. It is attached to the upper surface of the limbus, commencing near the angle formed by the membrane of Reissner, and extending over the organ of Corti, to be attached, perhaps, at its free margin to the edge of the cuticular membrane or Hensen's cells. Be this as it may, in sections this attachment, even if it does

exist normally, is broken, and the membrane is usually to be observed curled up at its edge—rolled up somewhat as a scroll might be, in the direction away from the organ of Corti. In section the lower margin of the membrane shows a double contour or hem.

The *cochlear nerve* passes along a central canal in the axis of the modiolus. It gives off a spiral lamina of nerve fibres, which passes through the osseous spiral lamina to be distributed to the organ of Corti. In its course through the osseous spiral lamina, or just at its point of entrance to it, it bears upon it the *spiral ganglion* (Fig. 162, *b*), a somewhat oval or round (in section) collection of nerve cells, which winds spirally round the modiolus with the other structures. The cells are very similar to those on the posterior root of a spinal nerve, but are bipolar, the processes not being fused together before the nerve cell is reached. Each process is an axis cylinder one, and acquires a medullary sheath. The fibres coming from the ganglion leave the tympanic lip of the spiral lamina by foramina beneath the inner hair cell layer, and at the point at which they pass beyond the connective tissue on which the epithelium rests they lose their medullary sheath, and are continued as naked axis cylinders. These split up into finer strands of fibrillæ, which are distributed as follows: Some of them change from radial to spiral in direction beneath the row of inner hair cells, and here constitute what is known as the *inner spiral strand* (Fig. 164, *o*), the fibrillæ of which are cut transversely in vertical section, and so appear as dots. From these a delicate network of filaments passes upwards to invest the bases of the inner hair cells with a kind of nervous nest. Other strands of fibrillæ are continued outwards between the limbs of the inner rods of Corti, and as they cross the tunnel one of them becomes spiral in direction, and constitutes the *spiral strand of the tunnel*. The others pass on between the limbs of the outer rods, and form four outer *spiral strands* beneath the rows of outer hair cells; and from these strands seen in section as dots, filaments proceed which form nervous nests to receive the bases of the outer hair cells. There are thus six spiral strands in all.

Examine the following specimen:—

(1,) *V. S. Cochlea of guinea-pig, stained with picro-carmin.* B. (Figs. 161, 162, 163, 164.)

The cochlea requires to be cut in celloidin, as the organ of Corti is much too delicate to bear the removal of paraffin in

FIG. 163.

V.S. COCHLEA OF GUINEA-PIG, STAINED WITH Picro-CARMINE $\times 300$.

- a.*—Crista spiralis.
- b.*—Sulcus „
- c.*—Membrana tectoria.
- d.*—Osseous spiral lamina.
- e.*—Membranous spiral lamina.
- f.*—Organ of Corti.
- g.*—Bone of outer wall.
- j.*—Nerve fibres.
- l.*—Ligamentum spirale.
- M.*—Scala vestibuli.
- N.*— „ media.
- P.*— „ tympani.

FIG. 164.

V.S. ORGAN OF CORTI (SEMI-DIAGRAMMATIC), STAINED WITH Picro-CARMINE $\times 600$.

- a.*—Crista spiralis.
- b.*—Sulcus „
- c.*—Membrana tectoria.
- d.*—Part of osseous spiral lamina.
- e.*—Membranous „ „
- f.*—Rods of Corti.
- g.*—Outer hair cells.
- h.*—Deiters' cells.
- b.ⁱ*—Vas spirale.
- b.ⁱⁱ*—Ligamentum spirale.
- j.*—Nerve fibres.
- k.*—One of the outer spiral strands.
- o.*—Inner spiral strand.
- m.*—Hensen's cells.
- n.*—Cells of Claudius.

(*Erratum.*—Of the three *b*'s in the figure, the middle should be *bⁱ* and the outer *bⁱⁱ*.)

Fig. 163.

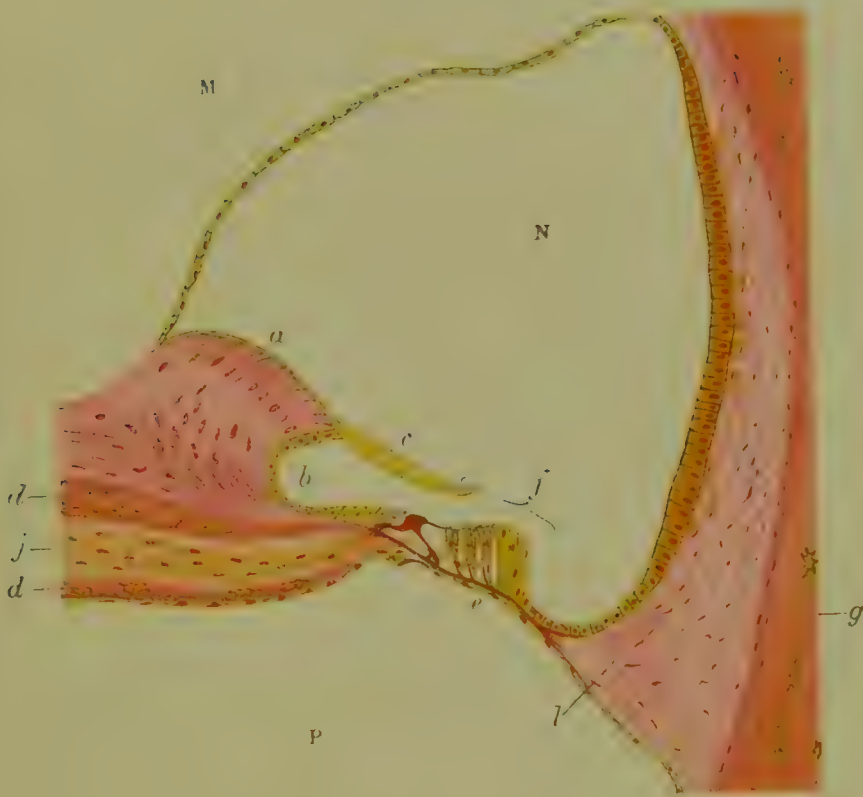
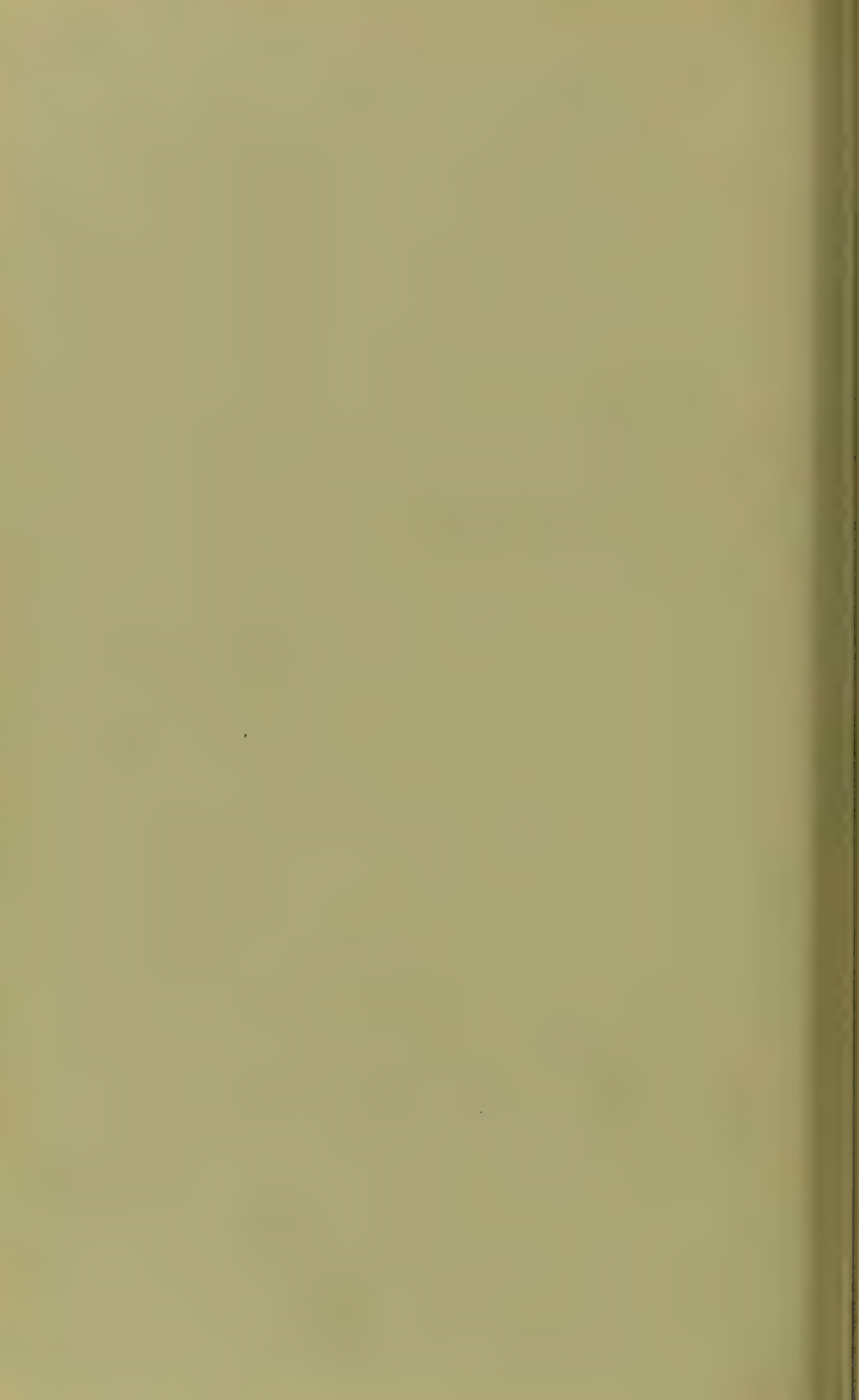


Fig. 164.





mounting the sections; with celloidin the removal of the embedding medium is not a necessity, as it is transparent in thin layers. Under the low power, note the general arrangement of parts (*Fig. 162*). Find the central channel in the modiolus containing the cochlear division of the auditory nerve (*a*), and note that the latter gives off a branch (as it appears in section) to the osseous spiral lamina on each side as it passes it. Find, on the branch, the spiral ganglion (*b*), cut in transverse section. Select one of the sections of the projecting osseous spiral lamina, and follow the nerve on in its course till the organ of Corti, on the membrana basilaris (*c*), is reached. Note the two lips of the limbus laminæ spiralis: the vestibular, forming a well-marked crest, the crista spiralis; and the tympanic, a thinner point to which the membrana basilaris is attached. From the top of the crista (*Fig. 163, a*), observe the membrana tectoria (*c*), projecting over the organ of Corti (*f*), and the sulcus spiralis (*b*), between the two lips. Under this power, note the deeply-stained rods of Corti, and the projection to the outside caused by the cells of Hensen. The details of the structure will be better seen under the high power. Follow the epithelium on to the spiral ligament (*l*), and note the breadth of the layer where it covers the stria vascularis along the outer wall of the canalis cochlearis. Trace it onwards into the membrane of Reissner, and so back to the crista (*a*). The connective tissue of the crista and spiral ligament is easily seen in this general view to be merely a development of the periosteum lining the cochlea in these situations, and is readily distinguished from the bone by its lighter staining. Before changing the power, note the scala vestibuli (*Fig. 162, v*), above the scala media (*M*), and the scala tympani (*T*) below it. Select the organ of Corti, which appears to show the most. It will be found usually that there is considerable variation in the sections on either side of the modiolus, as the structures are so readily spoilt during preparation, cutting, or mounting. Under the high power (*Fig. 164*), examine the structures already described. The crista spiralis, the membrana tectoria, the sulcus, the basilar membrane, and spiral ligament, are all very distinctly seen, as are also the rods of Corti. The latter form a well-defined landmark, which it is well to determine early in the examination. The characters of the hair cells and Deiters' supporting cells cannot usually be so clearly seen, nor the distribution of the nerve fibrils after they have left the osseous spiral lamina. These structures require

great care and skill, and special methods to reveal them, and can scarcely be relied upon in class specimens. The cells of Hensen can usually be quite easily made out, and the epithelium traced onwards round the canalis cochlearis, as indicated for low power examination.

THE OLFACTORY MEMBRANE.

The mucous membrane of the nasal cavity is divisible into two parts, an *olfactory* and a *respiratory*; the former of which covers the upper and part of the middle turbinated bones, and the upper third of the septum. Below this the cavity is lined by the respiratory or Schneiderian membrane.

The **respiratory membrane** is not unlike that of the trachea and large bronchi. The epithelium is of the stratified ciliated variety, with goblet cells here and there, and rests on a basis of subepithelial connective tissue, in which are found numerous small branched mucous glands.

The **olfactory membrane** is, however, specially modified. It is thicker than the respiratory, and presents a somewhat yellowish colour, due to the presence of pigment in the epithelium. In a vertical section the following structures are to be made out, and it will be noted that there is a considerable resemblance between the arrangement of the epithelial elements and that of the cells of the maculæ acusticæ, as already described. At the surface is to be seen a row of cylinder cells placed as usual palisade-wise, with their nuclei in their lower extremities; beneath this row a well marked nuclear layer, the whole resting on a connective tissue basis traversed by the non-medullated fibres of the olfactory nerve, which are here arranged in a plexiform manner. The *cylinder cells* possess a body of much the same shape as that of those already described in the macula, the free extremity forming part of the surface of the layer, while the lower end contains a somewhat oval nucleus; but the cell is prolonged at this end into an irregular process, which becomes lost to view between the nuclei of the nuclear layer. These cylinder cells are continuous with the ciliated cells of the respiratory region, and according to many authorities are not concerned with the sense of smell. The *rod cells* of the nuclear layer each contain a well marked spherical nucleus, surrounded by a very small amount of cell substance. The latter is prolonged upwards as a rod shaped

process, between the cylinder cells, and as such reaches the surface, where it abruptly terminates. There is also a central process which frequently shows varicosities upon it similar to those of a nerve fibril, which passes down between the nuclei of the layer, and, like that of the cylinder cell, is not very distinctly traceable further. The nuclei of these rod cells vary in position, some being placed near to the peripheral end of the cell, or at least close to the lower ends of the cylinder cells, while others are near to the connective tissue basis, and others are intermediate in position. A third kind of cell, "the basal cell," is also described. It is said to be irregular and branched, to possess an oval nucleus, and to lie immediately next to the dermis.

In some animals, such as the frog, there projects from the free end of the rod cells a brush of fine hair-like processes, which appear to be capable of ciliary movement.

The olfactory mucosa, like the respiratory, possesses glands, the alveoli of which lie in the subepithelial connective tissue, while their ducts open on the surface. The glands are tubular rather than saccular; they are smaller than those of the respiratory region, and they are more frequently serous than mucous. They are called the glands of Bowman.

It is not as yet very clearly decided as to whether the cylinder cells, the rod cells, or both, are to be regarded as the peripheral terminations of the olfactory nerve. If the structure of the membrane is analogous to that of the organ of taste (*i.e.*, the taste buds), it would appear that the rod cells are to be regarded in this light; on the other hand, if it corresponds with that of the maculæ acousticæ, the cylinder cells should be so regarded. According to some the fibres of the olfactory nerve are connected with the rod cells, and not with the cylinder cells; according to others, both kinds of cells are in relation with the nerves. But it is to be borne in mind that anatomical continuity is not necessary for functional continuity.

The balance seems on the whole to be in favour of the rod cells being the nerve terminations. It may be noted that the nuclei of the rod cells are spherical, not oval, as those of the cylinder and basal cells are; and, in addition, their reaction to staining reagents is not the same. They stain much less deeply with hæmatoxylin (Foster).

Examine the following specimen :—

V. S. Olfactory membrane of young mouse, stained with borax-carminé. B.

It is better to use the whole head as indicated in the "Appendix."

Under the low power examine the general arrangement of the parts, as already described on page 171. Compare the olfactory and respiratory portions of the epithelium lining the basal cavities, as far as their thickness is concerned ; and even under this power note the presence of the cylinder cells in the former, arranged as in a palisade. Note, too, the peculiar colour of the cells, due to the pigment they contain. Study the subepithelial connective tissue, and observe the presence of numerous glands in both parts of the membrane. Under the high power examine especially the olfactory epithelium. The cylinder cells are very easily distinguished and their characters made out ; the rod cells not so easily ; frequently little but the nuclei of this layer can be seen. Observe that there are no cilia projecting from the surface of the epithelium in the olfactory region. Now pass to the respiratory portion, and recognise the character of the lining, comparable with that of the trachea ; trace the cylinder into the ciliated cells.

APPENDIX TO CHAPTER XVI.

METHODS OF PREPARATION.

1. V.S. Cornea.—Excise the cornea; harden in Müller and spirit, cut in gum, stain in picro-carmin or hæmatoxylin, and mount in Farrant or balsam; or stain in bulk in borax-carmin, and cut in paraffin. The cornea of the eye of an ox or sheep is very suitable.

2. Cornea, stained with gold chloride.—(See text, p. 483.)

3. Cornea, stained with nitrate of silver.—(See text, p. 485.)

4. Eyeball of ox.—Obtain two or three eyes of sheep or ox, fresh. With a razor incise the eye so as to admit fluid into the cavity behind the lens, and into the anterior chamber. Harden for two weeks in excess of Müller's fluid, frequently changed; then commence to add spirit, and finally preserve in the latter. The following parts of the eye may be prepared from it: (*a*,) **Cornea.** (See 1); (*b*,) **Corneo-sclerotic junction.** Divide the eyeball equatorially into an anterior and posterior half. After removing the lens, divide the former by a crucial, antero-posterior section into four parts, taking care not to displace the delicate retina, which is very liable to become separated. Divide one of the quarters again, so as to get a suitable piece for section cutting. It may be cut quite well in gum if care is taken not to rupture the ligamentum pectinatum, and to preserve the retina; but, if preferred, it may be stained in bulk in borax-carmin, and cut in paraffin; (*c*,) **Sclerotic, choroid, and retina.** Cut out a piece of the eyeball near the equator, showing the three layers. Stain in bulk in borax-carmin, and cut in paraffin. The layers will not hold together if the sections are cut in gum; (*d*,) **V.S. Entrance of optic nerve.** Cut the posterior half, or less, of the eye from before backwards, so as to show the optic nerve in longitudinal section, and its point of entrance into the eyeball. The sections can be cut in gum and stained subsequently. Here again the retina requires care when the sections are placed in water after cutting. It is seen in the water as two delicate horns projecting from the middle of the concavity of the cup; (*e*,) **V.S. Retina.** Cut out a piece of retina, stain in bulk in borax-carmin, and embed in paraffin; or cut in gum, and stain subsequently in picro-carmin or hæmatoxylin; (*f*,) **T.S. Optic nerve.** Cut in gum, and stain in hæmatoxylin; mount in balsam.

5. The lens.—Remove the lens in its capsule from the eye of a recently-killed cat or rabbit. Harden in Müller's fluid, cut in gum; stain in picro-carmin or hæmatoxylin, and mount in Farrant or balsam. For **developing lens** use the head of a chick, hardened in Kleinenberg's picric acid solution. Stain in bulk in borax-carmin, and cut in paraffin.

6. The retina. As in 4c; or use the whole of the posterior half of the eye of a cat or rabbit; or harden in 1 per cent. osmic acid, and treat in a similar way. By cutting the three layers together, the retinal pigmented epithelium is retained in its relation to the rods and cones. For showing the *influence of light on the pigment cells*, use the eyes of the frog. Keep one frog in the dark for twelve hours, and expose another to light under a bell jar for a similar period. Kill the first, and transfer its head to the hardening fluid without admitting light. Spirit, or osmic acid (1 per cent. solution), may be used, but the fluid should be in excess, and light should be excluded while the tissues are being fixed. At the end of forty-eight hours, dissect the eyes from the head, and preserve in spirit. Stain in bulk in borax-carminé, and cut antero-posterior sections of the whole eye in paraffin. The eyes of the second frog may be treated in the same way, but are not to be kept in darkness during hardening. Retinæ of frog prepared in this way give very beautiful results.

7. Y.S. Retina stained with nitrate of silver (Golgi).—Golgi's method of staining nerve cells with nitrate of silver will be described more particularly in the chapter on the "Central Nervous System," but its application to the eye may be mentioned here. By treating with this reagent, the nerve cells of the different layers with their branching processes are stained black. Stirling gives the following method as yielding good results:—

(1.) Place the retina and sclerotic in weak osmico-bichromate fluid (osmic acid 1 per cent., 2 parts; bichromate of potassium 3 per cent., 20 parts), for twenty-four hours.

(2.) Transfer to 1 per cent. silver nitrate for twenty-four hours

(3.) Again, for twenty-four hours, as in (1).

(4.) " " " in silver nitrate.

Harden in alcohol; cut in paraffin; mount in balsam, but without a cover-glass.

8. Isolated retinal elements.—Place in dilute alcohol (1 in 2 of water) for a day or two, using a small quantity of the fluid. Stain in picro-carminé, tease, and mount in glycerine or Farrant.

9. Y.T.S. Human eyelid.—Harden in Müller and spirit; cut in gum, stain in picro-carminé or hæmatoxylin, and mount in Farrant or balsam; or stain in bulk in borax-carminé, and cut in paraffin.

10. Y.S. Cochlea of guinea-pig.—The cochlea of the guinea-pig is a very convenient one, as the temporal bone is very easily separated from the rest of the skull, and the cochlea is contained in a "tympanic bulla," so that this part of the osseous labyrinth is readily isolated. Remove the temporal bone, find the bulla, and clip away the shell exposing the cavity containing the cochlea. With a needle, puncture one or more of the spiral turns, in order to admit the reagent, and harden in Müller's fluid; then decalcify in chromic and nitric fluid, and transfer to spirit to complete the hardening. Cut in celloidin; stain in picro-carminé; clarify in origanum oil, and mount in balsam; or the tissue may be stained in bulk in borax-carminé before embedding.

11. Y.S. Olfactory membrane.—Use the head of a young mouse. Harden in Müller's fluid and spirit, decalcify in chromic and nitric fluid, stain in bulk in borax-carminé, and cut in paraffin.

CHAPTER XVII.

*THE CENTRAL NERVOUS SYSTEM.**THE SPINAL CORD, THE MEDULLA, AND BRAIN.*

THE SPINAL CORD.

THE spinal cord is traversed throughout by the *central canal*, which is immediately surrounded by the *grey matter*; the latter is invested with the *white matter*, and the whole is enveloped in three membranes, the *dura*, the *arachnoid*, and the *pia mater*. The *dura mater* surrounding the cord differs from that enveloping the brain, in that it does not constitute the periosteum of the bony canal or cavity in which it lies. It hangs loosely around the cord, being attached, on the one hand, to the bodies of the vertebræ in front of it, and, on the other, to the spinal nerves, to which it gives connective tissue sheaths. The *dura* is a dense fibrous membrane, whose chief function is a protective one. The *arachnoid* membrane is virtually the inner layer of a serous sac investing the central nervous system, and lying between the *dura* and *pia*. The sac is lined by simple squamous epithelium, the outer layer of which is applied to the inner surface of the *dura*, but the inner is separated from the *pia* by a reticulum of delicate connective tissue. There is thus a subdural space, which is virtually the cavity of the serous sac and a sub-arachnoid space, which is the interval between the visceral epithelial layer of the sac and the subjacent *pia*—an interval bridged across by delicate strands of connective tissue. In other words, the space between it and the *pia* is traversed by numerous bridges of connective tissue, which split it up accordingly into inter-communicating loculi, and as these loculi are lined by simple squamous epithelium, we may consider the sub-arach-

noid space a second serous cavity. The sub-arachnoid space is in communication with the ventricles of the brain, and contains cerebro-spinal fluid. The pia mater lies immediately upon the cord, and corresponds with the fibrous investment of organs generally. It is very delicate, and is made up of white fibres without any inter-mixture of elastic. It is vascular, and sends septa bearing blood-vessels into the substance of the cord for its nutrition. Immediately subjacent to the pia is a narrow layer of neuroglia, which has sometimes been described as part of it.

The white and grey matter of the cord is divided into lateral halves by the *anterior* and *posterior median fissures*, which are readily recognisable along its whole length with the naked eye. The "fissures" extend deeply, but they do not meet, a *commis-sure* of grey; and another of white matter being thus left between the two halves. The anterior fissure is comparatively broad and shallow, the posterior is narrow and prolonged more deeply. In fact, the posterior cannot in strictness be regarded as a fissure. It consists merely of an undivided septum passing in from the pia mater; the anterior fissure, on the other hand, is an actual one, the pia mater passing down one side and up the other.

The grey commissure (in contact behind with the posterior septum, but separated from the anterior fissure in front by the white commissure) contains the central canal and unites the two crescentic (in transverse section) columns of grey matter, much as the cross-bar of the letter H unites the two side pieces. These crescents have their concavity directed outwards, and possess each an *anterior* and *posterior horn*. The former is blunt and short, and from it several nerve bundles pass, to leave the cord as the anterior nerve root; the latter is long and pointed, and is continued to the surface as one bundle, which leaves the cord as the posterior nerve root. In the dorsal region of the cord a small *lateral horn* makes its appearance; and there are considerable variations in the shape of the grey matter and the amount of it relative to the white at different levels.

The grey matter, both of the anterior and posterior horns, consists of nerve fibres, nerve cells, and a sustentacular tissue, peculiar to the central nervous system, termed neuroglia, of epiblastic origin, and connected with the epithelial lining of the central canal. Many of the nerve fibres are non-medullated; they frequently, however, are very fine medullated ones. In addition the grey matter contains an exceedingly complicated

interlacement of fibrils, the result of repeated subdivision of the peripheral processes of the ganglion cells.

The white matter surrounds the grey, and is divisible into three well marked areas: (*a*,) Between the points of exit of the anterior nerve roots, where it forms the *anterior columns*, one on either side of the anterior median fissure; (*b*,) between the points of exit of the anterior and posterior nerve roots, where it forms the *lateral columns*; and (*c*,) between the posterior nerve roots, where it forms the *posterior columns*, one on either side of the posterior median septum.

But these columns are themselves further subdivided. Thus, a well marked septum from the pia mater subdivides the posterior column on either side, especially in the cervical region, into a *postero-external* and *postero-internal* column, and there are other subdivisions which will be referred to later. Many of these are not obvious anatomically in a section of an adult healthy cord, but are picked out in cases of disease. Thus we have ascending degeneration of sensory tracts, and descending degeneration of motor ones, both of which may be induced artificially by hemisection of the cord a week or more before it is required. When treated by Weigert's process the degenerated parts are comparatively unstained owing to the disappearance of the myelin.

Sections of embryo cords when stained by Weigert's method also give valuable results in differentiating the tracts, in some of which the medullary sheath appears later than in others. Thus, the pyramidal tracts may be shown in the fœtus.

The white matter, unlike the grey, does not contain nerve cells. It is composed of longitudinally running medullated nerve fibres of different sizes, which are, however, destitute of a sheath of Schwann, and which, it is also stated, want the nodes of Ranvier and internodal nuclei (Stewart). Between the nerve fibres is to be found the supporting neuroglia, which is in relation with the basal processes of the ciliated epithelium lining the central canal, through its continuity with that of the grey matter itself. The white commissure is composed of medullated fibres passing from one anterior column to the other.

It will now be well to consider the structure of the white and grey matter of the cord in a little more detail.

The white matter of the cord: its structure and arrangement.—The white matter is composed of longitudinally running medul-

FIG. 165.

T.S. SPINAL CORD (HUMAN), DORSAL REGION, STAINED WITH ANILINE
BLUE-BLACK $\times 10$.

- a.*—Anterior median fissure.
- b.*—Posterior median septum.
- c.*—Anterior horn of grey matter.
- d.*—Anterior nerve root.
- e.*—Posterior horn of grey matter.
- f.*—Clarke's column of nerve cells.
- g.*—Posterior nerve root.
- h.*—Pia mater.
- i.*—Central canal of cord.
- j.*—Prolongation inwards from pia mater.
- k.*—Lateral horn.
- l.*—White matter of cord.

FIG. 166.

T.S. SPINAL CORD (HUMAN), LUMBAR REGION, STAINED WITH ANILINE
BLUE-BLACK $\times 10$.

- a.*—Anterior median fissure.
- b.*—Posterior median septum.
- c.*—Anterior horn of grey matter.
- d.*—Anterior nerve root.
- e.*—Posterior horn of grey matter.
- g.*—Posterior nerve root.
- f.*—Fibres of internal radicular fasciculus of posterior nerve root.
- h.*—Pia mater.
- i.*—Blood-vessel.
- k.*—Central canal of cord.
- l.*—White matter.

Fig. 165.

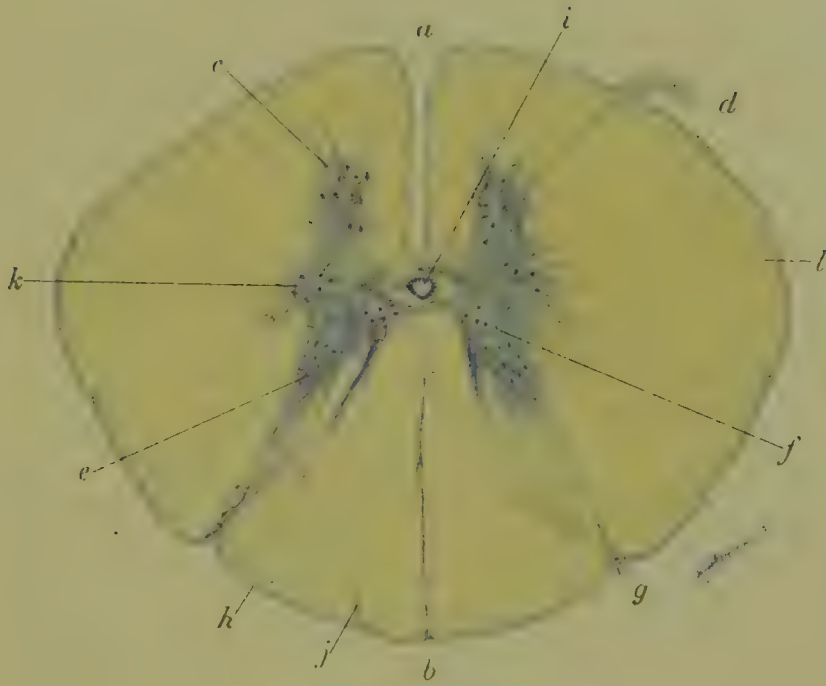
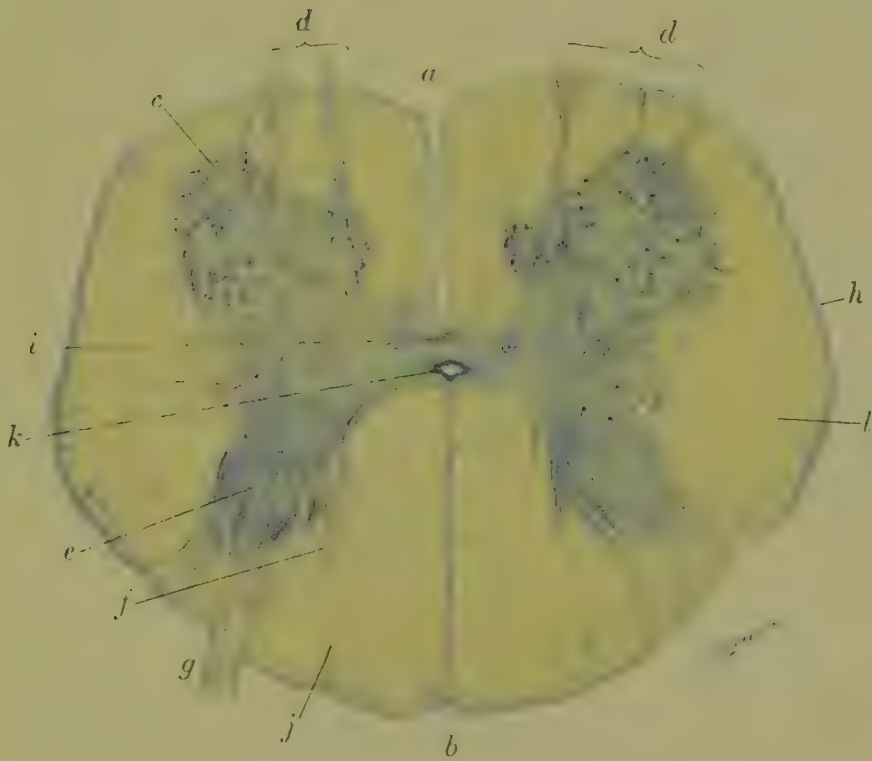


Fig. 166.





lated nerve fibres supported by neuroglia. The nerve fibres do not possess a sheath of Schwann. They vary very much in size in any part of the section selected, and in some strands, too, are either larger or smaller than in others. Thus, they are smallest in the postero-median (a sensory tract of fibres) and largest in the direct and crossed pyramidal (motor tracts). The neuroglia consists of large branched cells (of epiblastic origin), which send processes between the nerve fibres, which also receive support from the processes derived from the deep surface of the pia mater. The delicate branches of the neuroglia cells form a very complicated interlacement of fibrils, which are embedded in a homogeneous ground substance. Each nerve fibre is thus surrounded by a tubular sheath of neuroglia. Occasionally, *outlying* nerve cells are found amongst the white fibres immediately outside the grey matter.

The white matter of the cord is divided physiologically into certain tracts. The anterior column is divisible into the *direct pyramidal tract* immediately bounding the anterior median fissure, and the *anterior ground bundle* or *anterior radicular zone*. The direct pyramidal tract passes into the medullary pyramid of the *same* side. The posterior column is divisible into a *postero-median column* or *column of Goll*, and a *postero-external* or *column of Burdach* or *posterior radicular zone*. The

lateral column of the cord, that part of it between the anterior and posterior nerve roots of either side, is subdivided into the *antero-lateral mixed tract*, consisting of both ascending and descending (*i.e.*, afferent and efferent) fibres, the *lateral limiting layer*, the *crossed pyramidal tract*, and the *direct cerebellar tract* (see *Fig. W*).

Of these tracts of the white matter of the cord some are afferent and some are efferent, and transverse section of the cord is followed by ascending degeneration of the afferent, and

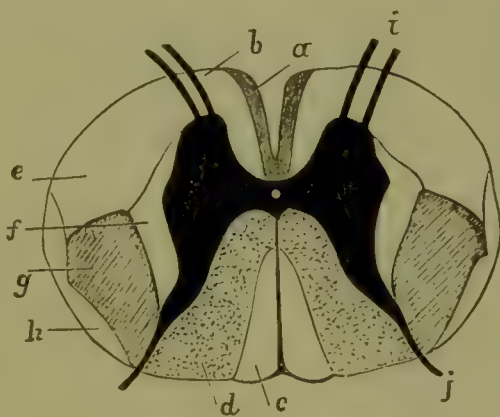


Fig. W.—*a*, Antero-internal column (direct pyramidal tract); *b*, antero-external column (ground bundle); *c*, postero-internal column (column of Goll); *d*, postero-external column (column of Burdach); *e*, anterior mixed zone of lateral column (including ascending and descending antero-lateral tracts); *f*, lateral limiting tract; *g*, crossed pyramidal tract; *h*, direct cerebellar tract; *i*, anterior nerve root; *j*, posterior nerve root.

descending of the efferent; some of the tracts, on the other hand, do not degenerate.

The direct and crossed pyramidal tracts are efferent, and are connected with the motor areas of the cortex of the cerebrum; they undergo descending degeneration after section of the cord. Part also of the antero-lateral mixed tract degenerates, and is therefore termed the *antero-lateral descending tract*. The columns of Goll, on the other hand, the direct cerebellar, and certain fibres of the antero-lateral mixed tract, termed in consequence the *ascending antero-lateral tract*, degenerate above the point of lesion. The anterior ground bundle, the postero-external column, and the lateral limiting layer do not degenerate.

The grey matter of the cord.—As we have seen, the grey matter consists of a basis formed of the interlacement of fine fibrils derived from the protoplasmic or peripheral processes of the nerve cells, of nerve cells themselves, and of neuroglia. Fine medullated nerve fibres also are to be seen entering the grey matter to join nerve cells or to split up into fibrillar divisions of their axis cylinder, and terminate in the common network. The nerve cells are found both in the anterior and posterior horns. In the anterior they are large, multipolar, ganglion cells, with one axis cylinder process and many peripheral protoplasmic ones, which break up to form the interlacement of fibrils referred to. In the posterior horn the cells are smaller and fusiform. The posterior horn is narrow and pointed in contrast to the anterior, which is short and broad. Moreover, it is invested with a cap of neuroglia, which is termed the *substantia gelatinosa* of Rolando. The neuroglia contains a few fusiform nerve cells.

Arrangement of nerve cells.—The grey matter of the cord undergoes enlargement opposite the origin of the larger strands of nerves, and is thus especially in preponderance in the cervical and lumbar regions. In the *conus medullaris* the grey matter makes up nearly the whole of the substance of the cord, being surrounded with very narrow strands of white matter. The nerve cells are not scattered indefinitely throughout the grey matter, but are arranged in certain well defined strands. There are four chief subdivisions: (1,) Those of the anterior horn; (2,) those of the posterior horn; (3,) the intermedio-lateral column; (4,) Clarke's column.

The cells of the anterior horn are further subdivided into four groups according to the position they occupy. These are: (a,)

inner or median; (*b*,) anterior; (*c*,) antero-lateral; (*d*,) postero-lateral. As already stated, these cells of the anterior horn are large, multipolar, ganglion cells. The cells of the posterior horn are more scattered, and are small and fusiform. The cells of the intermedio-lateral tract form a distinct strand in the dorsal part of the cord, but in the cervical and lumbar fuse with those in the anterior horn. The vesicular column of Clarke is found to the inner side of the root of the posterior horn. It is not, however, continuous throughout the cord. The main portion is found in the dorsal region from the level of the seventh or eighth cervical nerve to the second or third lumbar; the cervical *nucleus of Stilling*, in the cervical region, and the sacral nucleus named after the same observer in the sacral region may be regarded as outlying portions of the same column.

Examine the following specimens:—

(1,) *T. S. Spinal cord, human (dorsal region), stained with Weigert's hæmatoxylin, or with aniline blue-black. B. (Fig. 165.)*

It will be convenient to suppose the specimen is stained by Weigert's method. Under the low power note the different staining of the grey and white matter. The grey is a brownish yellow, and is thus very distinctly demarcated from the white, which is stained a slate blue. Note that the pia mater is affected in the same way as the grey matter, and that its processes dipping into the periphery of the white matter are thus readily followed. Note first the central canal of the cord (*i*); that it is nearer the anterior than the posterior surface; and that it is placed in the grey commissure. Compare the two fissures, anterior and posterior. The former (*a*) is a true one, its two sides bounding a space; it is comparatively shallow and broad, whereas the latter (*b*) is merely a septum of connective tissue derived from the pia mater, which, however, penetrates deeply. Observe the grey and white commissures respectively. They are easily differentiated from each other in a Weigert specimen; the anterior lies at the bottom of the anterior median fissure, and being composed of white matter is stained with the hæmatoxylin; the grey commissure encloses the central canal, connects the two lateral crescents of grey matter, and like them is stained a brownish yellow. Compare the anterior and posterior horns of

FIG. 167.

T.S. SPINAL CORD (HUMAN), UPPER CERVICAL REGION, STAINED WITH ANILINE BLUE-BLACK $\times 10$.

- a.*—Anterior median fissure.
- b.*—Posterior median septum.
- c.*—Anterior horn of grey matter.
- e.*—Posterior " "
- f.*—Internal radicular fasciculus of posterior nerve root.
- g.*—Posterior nerve root.
- h.*—Pia mater.
- j.*—Septum from pia mater.
- k.*—Network of grey matter.
- l.*—White matter of cord.
- m.*—Central canal of cord.
- n.*—Nerve cells of anterior horn.

FIG. 168.

T.S. SPINAL CORD (HUMAN), LOWER CERVICAL REGION, STAINED WITH ANILINE BLUE-BLACK $\times 10$.

- a.*—Anterior median fissure.
- b.*—Posterior median septum.
- c.*—Anterior horn of grey matter.
- d.*—Anterior nerve root.
- e.*—Posterior horn of grey matter.
- f.*—Internal radicular fasciculus of posterior nerve root.
- g.*—Posterior nerve root.
- h.*—Pia mater.
- i.*—Anterior white commissure.
- j.*—Septum of pia mater marking off internal from external subdivision of posterior column.
- k.*—Network of grey matter.
- l.*—White matter of the cord.
- m.*—Central canal.
- n.*—Nerve cells in anterior horn.

Fig. 167.

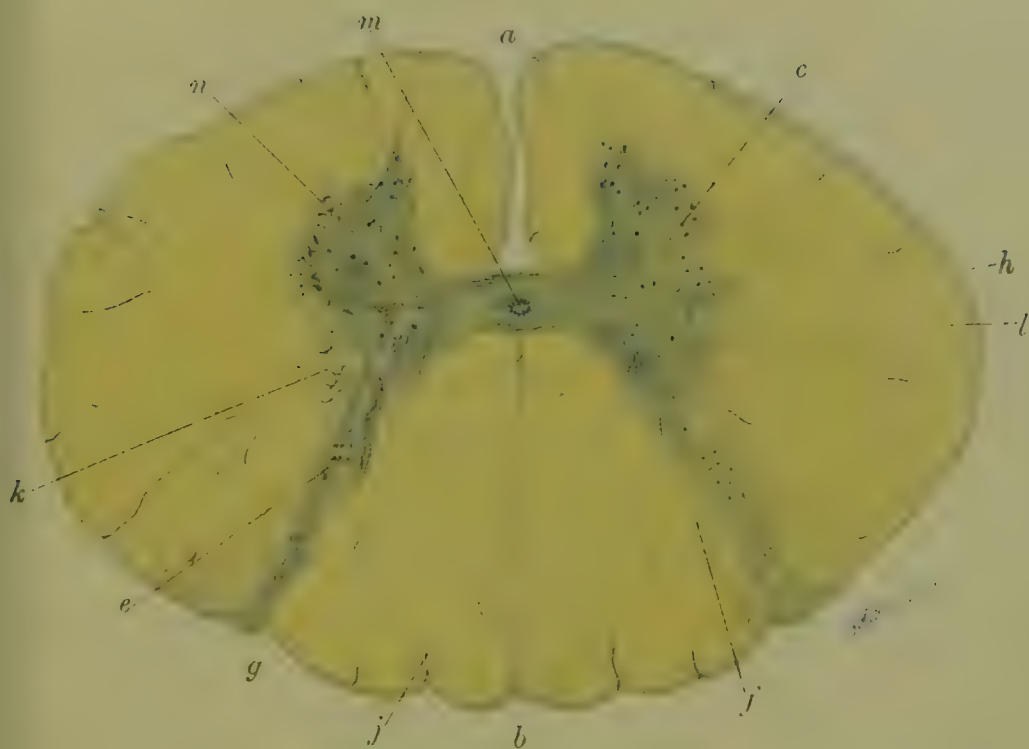
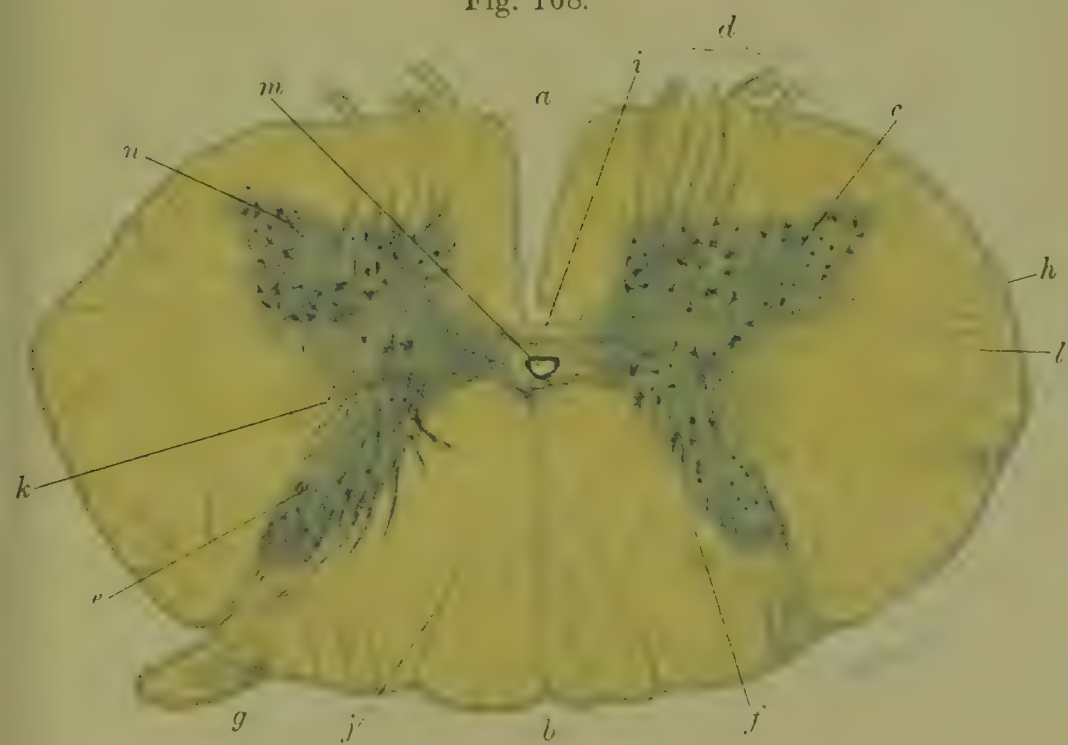


Fig. 168.





the crescents of grey matter. The anterior (*c*) is short and broad, and does not reach the surface, and its nerve root leaves it in several bundles; the posterior (*e*) is long and pointed, and the nerve root is undivided. Look, too, for the somewhat homogeneous cap of neuroglia covering the posterior horn, the *substantia gelatinosa* of Rolando. Observe, however, in this the dorsal region, that there is an intermediate lateral horn (*k*) containing a group of nerve cells, the intermedio-lateral tract. In the cervical and lumbar regions these cells fuse with those of the anterior horn. The cells of the posterior horn are small and scattered, and are not so easily seen under this power as those of the anterior. In both they are stained of a brownish colour, more deeply than the grey matter amongst which they are embedded. Look for Clarke's vesicular column (*f*) at the root of the posterior horn. It is situated at the inner side, at the point where the horn springs from the transverse grey commissure. Note the round or oval shape of the collection, and the medullated fibres stained with the hæmatoxylin, which can be traced from it into the postero-external column of white matter.

Note the relative amount of white and grey matter; in this region the former is considerably in excess. Look for an indication of the subdivision of the posterior white columns into internal and external strands, by a process of pia mater (*j*), and observe that the outer or radicular zone is traversed by fibres of the posterior nerve root, which bend out into it before they enter the grey matter of the posterior horn. These fibres constitute the internal radicular fasciculus of the posterior nerve root. The outer fasciculus passes directly into the grey matter through the *substantia gelatinosa*. Under the high power find first the edge of the specimen, and study the appearance of a transverse section of the white matter as shown in *Fig. 56*, though there the stain used has been picro-carmin. In a Weigert specimen the pia mater (*a*) is yellow, and the transversely cut medullary sheaths of the nerve fibres are stained blue. Look for neuroglia cells (*c*) stained of a brownish yellow. Note their large size and the processes passing from them between the nerve fibres. Observe the very various sizes of the latter. The nerve fibres receive support both from the neuroglia and from the processes of pia mater, which thus come into continuity with each other as shown on the left hand of the figure. Now examine the grey matter. Find the anterior horn, and note the large multipolar nerve cells

in it. One of these stained with picro-carmin is shown in *Fig. 59, c*. Observe their large nuclei and well defined nucleoli. It is not always possible in a section to distinguish between the axis cylinder and peripheral processes. Note the fine medullated nerve fibres stained with the hæmatoxylin traversing the grey matter. Next study the nerve cells of the posterior horn and the substantia gelatinosa covering it. Note that the nerve cells are small and fusiform in shape, contrasting thus with the large branching stellate cells of the anterior horn. The substantia gelatinosa is formed of homogeneous looking neuroglia, containing, here and there, a few nerve cells, and traversed by the fibres of the posterior nerve root, which give it a striated appearance. Examine Clarke's column, and recognise the bipolar nerve cells, many of them here cut equatorially as they run in the long axis of the cord, intermediate in size to those of the anterior and posterior horns. The cells of the intermedio-lateral tract are more nearly related to the cells of the posterior horn, and are of the same size or a little larger.

Now find the central canal and note the tall, columnar, ciliated cells lining it. Observe that they terminate in pointed basal processes, which become lost in a layer of neuroglia, upon which the epithelium immediately rests. Examine the grey and white commissures; trace the fibres from Clarke's column into the postero-external column of white matter, and follow out the course of the internal radicular fasciculus of the posterior nerve root as far as possible.

(2.) *T. S. Spinal cord, human (lumbar region), stained with Weigert's hæmatoxylin, or with aniline blue-black. B. (Fig. 166.)*

(3.) *T. S. Spinal cord, human (upper cervical region), stained with Weigert's hæmatoxylin, or with aniline blue-black. B. (Fig. 167.)*

(4.) *T. S. Spinal cord, human (lower cervical region), stained with Weigert's hæmatoxylin, or with aniline blue-black. B. (Fig. 168.)*

In each of these sections note the distinctive characters of the level at which it is taken. In the lumbar cord observe that the proportion of grey to white matter is greater than it is elsewhere; also that Clarke's vesicular column is not seen. The intermedio-lateral tract also has disappeared. In the lower cervical region (*Fig. 168*) the subdivisions of the anterior nerve

roots are well seen, and the septum of pia mater separating the postero-internal and external columns is well marked (*j*). Note also in this figure the course of the internal radicular fasciculus of the posterior nerve root shown at (*f*).

THE MEDULLA OBLONGATA.

In the medulla oblongata the strands of white matter of the cord undergo rearrangement. The grey matter also changes in shape and disposition, and new portions of grey matter become added.

In the cord, as we have seen, the grey matter is completely surrounded by the white. In the brain, on the other hand, the white matter is enveloped by the grey. In the medulla the condition is intermediate, the grey matter coming to the surface in the floor of the fourth ventricle, as the posterior pyramids become separated to expose the central canal. It is not within the scope of the present work to follow out the rearrangement of matter, white and grey, in all its details, but the following short account may assist the student to recognise the different parts seen in transverse sections of the medulla. The appearances vary considerably, according to the level at which the section is made.

As we pass from the cord to the medulla or bulb, the "decussation of the pyramids" takes place, whereby the crossed pyramidal tract of the one side passes over to join the direct pyramidal tract of the anterior column of the other, the two together forming the *anterior pyramids* (one on either side of the anterior median fissure of the medulla). The rest of the anterior column, namely, the antero-external division, passes, as we shall see, more deeply to form part of the *formatio reticularis*.

By the crossing over of the crossed pyramidal tract to the anterior column of the opposite side, the tip of the anterior horn of grey matter becomes "amputated," and is found in the medulla isolated from the rest, and is here known as the *nucleus lateralis*.

The postero-internal columns of the cord are continued upwards into the medulla as the *posterior pyramids* (*funiculus gracilis*), while the postero-external columns form on each side the *funiculus cuneatus*, which joins with parts of the lateral column and posterior horn to form the *restiform body*. The

FIG. 169.

T.S. MEDULLA OF CAT, AT THE LEVEL OF THE DECUSSATION OF THE PYRAMIDS, STAINED WITH WEIGERT'S HÆMATOXYLIN $\times 10$.

- a.*—Anterior median fissure.
- b.*—Posterior "
- c.*—Caput of posterior horn.
- d.*—Commencing cuneate nucleus.
- e.*— " clavate "
- f.*—Head of anterior horn becoming isolated.
- h.*—Fibres of anterior pyramid.
- i.*—Central canal.
- l.*—Decussation at bottom of anterior fissure.
- i.*—Fibres passing across root of anterior horn.

FIG. 170

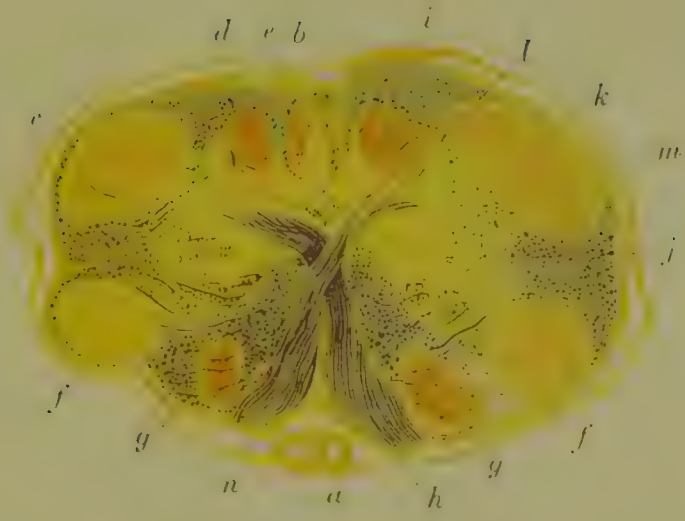
T.S. MEDULLA OF CAT, AT THE LEVEL OF THE LOWER PART OF THE OLIVE, STAINED WITH WEIGERT'S HÆMATOXYLIN $\times 10$.

- a.*—Anterior median fissure.
- b.*—Posterior "
- c, k.*—Caput of posterior horn.
- d.*—Nucleus cuneatus.
- e.*— " gracilis (clavate nucleus).
- f.*—Head of anterior horn, separated (nucleus lateralis).
- g.*—Olive.
- h.*—Anterior pyramid.
- i.*—Central canal.
- j.*—Commencement of formatio reticularis.
- l.*—Decussation.
- m, n.*—Membranes of the medulla.

Fig. 169.



Fig. 170.





latter may be regarded as consisting of the cuneate funiculus, the funiculus of Rolando (a prominence caused by the projection, towards the surface, of the caput of the posterior horn), the direct cerebellar tract and the arciform fibres; the whole constituting higher up the *inferior peduncle* of the cerebellum. The fibres of the direct cerebellar tract form a narrow band (in section), external to the funiculus of Rolando.

The rest of the fibres of the anterior and lateral columns of the cord takes part in the construction of the *formatio reticularis*, of which they seem to form the longitudinal strands. The rest of the formation is formed of the remains of the anterior horn, and of transversely passing fibres, which decussate in the middle line to form the raphé, and belong to the *arcuate system*. Of the arcuate fibres, some are superficial and some are deep. The *superficial* appear in the median fissure, probably being derived from the opposite side; and passing round the front of the olivary body, to which they thus afford a capsule, join the cerebellar fibres in the restiform body; the *deep*, having origin in the clavate and cuneate nuclei, sweep in curves towards the raphé, and cross to the other side.

The *olivary bodies*, projecting on each side between the anterior pyramids and the restiform body, contain the *corpus dentatum*, a flask-shaped fold of grey matter, containing many nerve cells embedded in neuroglia, and traversed by fine nerve fibres, many of which belong to the deep arcuate system and pass to the restiform body. There are also two *accessory olives*, one of which is below and internal, and the other above and external to the corpus dentatum. The root of the hypoglossal finds exit between the latter and the internal accessory olive.

But besides the amputation of the anterior horn and the appearance of the olivary bodies, other changes take place in the grey matter. The posterior horns project outwards, as we have seen, to form the funiculus of Rolando, which constitutes part of the restiform body; nearer the middle line the base, or root of the horn, gives rise to a mass of grey matter occupying the cuneate funiculus, and hence termed the *cuneate nucleus*, and next to the posterior fissure to another, forming the centre of the funiculus gracilis, and known as the *clavate nucleus*, *nucleus gracilis*, or *nucleus of the clava*. This disposition is further modified a little higher up by the separation of the posterior pyramids and the exposure of the central canal in the

floor of the fourth ventricle ; whereby the clavate nuclei, and also the cuneate on either side of them, are carried outwards from the middle line, and the grey matter of the floor of the ventricle becomes superficial. Here, near to the middle line, is found a portion of the root of the anterior horn persisting as the nucleus of origin of the hypoglossal nerve, which passes on either side of the median raphé, through the formatio reticularis, and leaves the cord to the inside of the olivary body. Other nuclei of grey matter, *e.g.*, the origins of the *vagus*, *spinal accessory*, and *glossopharyngeal* are also found in the floor of the fourth ventricle.

By far the simplest method of mastering these alterations in the disposition of parts is, however, to be found in the examination of the actual specimens. It is advisable to have a series of sections passing from below upwards, taken at different levels, and the less interval there is between the successive levels the more readily are the alterations followed. For obvious reasons, however, only a limited number of sections will be examined here.

Examine the following specimens :—

(1.) *T. S. Medulla of cat, through the commencement of the decussation of the pyramids, stained with Weigert's hæmatoxylin. B. (Fig. 169.)*

In this specimen it is not difficult to trace the relation of the parts to those of the spinal cord. Under the low power, it will be seen at first sight that the main point of distinction is the crossing of the pyramids (*h*). The decussation here at the bottom of the anterior median fissure is very beautifully seen, and apparently does not cause the deflection of the fissure, which is familiar to us in this region in the human subject. If the fibres are traced inwards on either side from the anterior column, they will be seen to cross over at the bottom of the fissure, and stream across the root of the anterior horn (*f*), which is thus separated ; and in doing this they form the commencement of the formatio reticularis (*j*). Now look at the posterior part of the section where instructive changes may be seen to be taking place. It is important, above everything, to recognise the beginning of these changes, or the tracing of them higher in the medulla becomes very difficult. Note the well marked enlargement of the caput of the posterior horn (*c*), and its approach to the surface of the medulla, where it causes a distinct projection, the funiculus of Rolando. Now look at the posterior

columns, and note their relation to the grey matter. From the inner side of the root of the posterior horn may be seen a growth of grey substance (*d*), which is extending into the postero-external column, and will in time form the nucleus cuneatus; and to the inner side of this note another projection of grey matter into the postero-internal column, to form the nucleus gracilis (*e*). Note the position of the central canal (*i*) and the surface differentiation of the postero-internal columns, to form the commencement of the posterior pyramids. There is little else to be noted in this section, except the character of the staining.

Under a higher power observe the transverse sections of the nerve fibres in the white matter, their medullary sheath stained blue; the fibres are seen in longitudinal section in the decussation, and as they pass through the anterior horn. Note the staining of the grey matter; the ground substance is a yellowish colour, the cells are brown, and fine medullated nerve fibres, stained blue, may be seen crossing through it, or here and there breaking up into fibrils.

(2.) *T.S. Medulla of cat through the lower end of the olivary body, stained with Weigert's hæmatoxylin. B. (Fig. 170.)*

Under the low power note the following points: The amputation of the head of the anterior horn is much more complete, and it is now found as an isolated mass, the nucleus lateralis (*f*). Between this and the anterior pyramid (*h*) has appeared the olivary body (*g*), but only the lower end of it has been cut, so that its flask shape, with the mouth of the flask turned inwards, is not seen. Nor can this characteristic shape be made out at any level, as well in the cat as in man, but it will be seen in the succeeding sections to be distinctly folded. The olivary body contains very well marked multipolar nerve cells, which are shown in the figure as reddish dots. The formatio reticularis (*j*) is still more in evidence, but no median raphé is yet distinguishable. Again, the caput (*c* and *k*) of the posterior horn is very readily to be made out on each side, and between the two the cuneate nucleus (*d*) and the nucleus gracilis (*e*) have become still more developed. Note again the position of the central canal (*i*), which is beginning to approach the posterior surface of the medulla.

(3.) *T.S. Medulla of cat, through the middle of the olivary*

FIG. 171.

T.S. MEDULLA OF CAT, THROUGH OLIVE, STAINED WITH WEIGERT'S
HÆMATOXYLIN $\times 10$.

- a.*—Anterior median fissure.
- b.*—Posterior „
- c.*—Caput of posterior horn.
- d.*—Nucleus cuneatus.
- e.*—Nucleus gracilis.
- f.*—Nucleus lateralis.
- g.*—Olivary body.
- h.*—Anterior pyramid.
- i.*—Central canal.
- j.*—Formatio reticularis.
- k.*—Spinal accessory nerve.
- k*¹—Nucleus of spinal accessory.
- l.*—Median raphé.

FIG. 172.

T.S. MEDULLA OF CAT, AT LEVEL OF COMMENCEMENT OF FOURTH
VENTRICLE, STAINED WITH HÆMATOXYLIN $\times 10$.

- a.*—Anterior median fissure.
- b.*—Posterior median fissure opening into central canal (*i*).
- c.*—Caput of posterior horn.
- d.*—Cuneate nucleus.
- d*¹—External cuneate nucleus.
- e.*—Nucleus gracilis.
- f.*—Nucleus lateralis.
- g.*—Olive.
- h.*—Anterior pyramid.
- i.*—Central canal, opening to form floor of fourth ventricle.
- j.*—Formatio reticularis.
- k.*—Spinal accessory nerve.
- k*¹—Nucleus of spinal accessory.
- l.*—Median raphé.

(*Erratum.*—Of the two *k*'s in the figure, the higher should be *k*¹,
as in *Fig. 171.*)

Fig. 171.



Fig. 172.



body (but below the level of the separation of the posterior pyramids at the *calamus scriptorius*), stained with Weigert's hæmatoxylin. B. (Fig. 171.)

The resemblance to the cord is by no means so obvious now, but the tracing of the parts presents no real difficulty, if the previous sections have been properly understood.

First identify the anterior pyramid (*h*) on either side of the anterior median fissure (*a*). This is easily done by noting their projection, their deeper staining (as they consist of white matter), and their distance from the central canal, which is now close to the posterior pyramids. Lying in close proximity to, but deeper than the anterior pyramids, recognise the convoluted folds of the olive (*g*) and the nerve cells, dotted through its grey matter; fine medullated fibres also are revealed by the reagent, crossing it from side to side. To the outside of the olive (*g*), endeavour to recognise the remains of the head of the anterior horn—the nucleus lateralis (*f*). At this level it is not, however, so easily recognisable as a distinct mass of grey matter as in the last section. Now pass to the posterior fissure (*b*), and work again to the front from that point. On either side of the fissure we naturally look for the funiculus gracilis with its now enlarged nucleus (*e*), and to the outer side of it the funiculus cuneatus, whose nucleus (*d*) has also become further developed. In either case, the white matter will be seen to form merely a shell to the grey. To the outside of the cuneate nucleus we find, as before, the head of the posterior horn (*c*), and working still further forwards, the nucleus lateralis (*f*) again comes into view. So far, the grey matter presents no great difficulty. Now look for two strands of white matter, which have been passed over in the regions just examined. Observe the narrow band (dotted in the figure) at the periphery of the medulla, bounding the posterior horn and nucleus lateralis. It represents the direct cerebellar tract of the cord, the fibres of which join the restiform body to form the inferior peduncle of the cerebellum. Within this is to be seen another strand of white matter, continuous with the shell covering the gracile and cuneate nuclei; it is, however, more distinctly seen in the next specimen.

Now find the central canal (*i*), and between it and the anterior median fissure (*a*), distinguish the median raphé (*l*) with the fibres, transverse and longitudinal, of the *formatio reticularis* (*j*), on either side of it. Immediately in contact with the raphé, the

longitudinal bundles of the formatio reticularis (cut here, of course, transversely) are very much in evidence.

Observe the spinal accessory nerve (*k*), passing from a nucleus of grey matter (*k*¹) in close proximity to the central canal, through the formatio reticularis, and finding exit from the medulla between the olive and nucleus lateralis. Before leaving the section study the arcuate fibres, superficial and deep, sweeping in front of and behind the olive to join the fibres of the direct cerebellar tract. Observe that the medulla is now roughly divisible into four areas externally on either side: (1,) the anterior pyramid; (2,) the olivary projection; (3,) the restiform body; (4,) the posterior pyramid. The point of exit of the accessory nerve indicates the line of separation between the olivary and restiform bodies; higher up, other nerves, in leaving the medulla, separate the olive from the anterior pyramid.

(4.) *T. S. Medulla of cat at level of commencement of fourth ventricle, stained with Weigert's hæmatoxylin. B. (Fig 172.)*

In this section, most of the points noted in the last are to be made out, and also some new ones. Note that here the central canal (*i*) has reached the surface, and that the posterior pyramids (*e*) have been thereby separated; so that the section is taken through the point of the calamus scriptorius, *i.e.*, at the level where the posterior pyramids diverge to expose the floor of the fourth ventricle. Observe, again, the white matter covering the cuneate nucleus and the caput of the posterior horn, and in it, note, what is a little difficult some times of demonstration, the *external nucleus of the funiculus cuneatus* (*d*¹). Anterior and external to this strand of white matter, note again the cerebellar strand, which receives the arciform fibres in the region of the nucleus lateralis (*f*). In both this specimen and the last, note the transversely running arciform fibres passing from the cuneate and gracile nuclei in bold curves towards the raphé (*l*), in which they cross to the other side.

THE CEREBRUM.

A convolution of the cerebrum consists of a cortical layer of grey matter, and a medullary of white; in this way exactly reversing the condition found in the cord, in which the grey matter forms a central column. The grey matter possesses a "molecular basis" consisting of fine processes of nerve and

neuroglia cells, and contains a large number of pyramidal nerve cells which are connected with fibres from the white medulla ; in fact, the cortex is shown to be permeated throughout with a complicated arrangement of fine medullated fibres, some of which run radially, some parallel to the surface of the convolution. A vertical section of the cortex shows the following layers (*Fig. 174, B*) : At the surface is the *pia mater* (*a*) ; beneath it (1,) the *superficial* or *molecular layer* of grey matter (*a*¹), consisting mainly of neuroglia ; (2,) the *layer of small pyramidal nerve cells* (*b*) ; (3,) the *layer of large pyramidal nerve cells* (*c*) ; (4,) the *layer of irregular cells* (*d*) ; and (5,) the *fusiform cell layer* (*e*) ; after which comes the white matter (*f*). The names of the layers to some extent indicate the characters of the cells found in them. The larger pyramidal cells, especially found in the cortex of the motor areas, are shown in *Fig. 59 (e)*, and the small at (*e*¹). They are pyramidal in shape, their apex pointing to the surface, their base being directed to the white matter with which they are connected by their axis cylinder process (*f*). They give off numerous peripheral processes which break up into still finer ones, and join the molecular basis. Each cell lies in a lymph space of its own which is in relation to a peri-vascular lymphatic channel. These are of the nature of a hollow jacket, surrounding a vessel, formed of two layers of epithelial cells with a sinuous outline—one applied to the vessel wall, and the other lining the space in the tissues in which it lies. One of these peri-vascular lymphatics is shown in *Fig. 66*, but only the outer layer of epithelium is shown. The irregular layer consists of a number of small branched, irregular, ganglion cells ; and the fusiform layer of spindle cells, which lie parallel with the surface of the convolution. Both these layers, (4,) and (5,) (*d* and *e* in *Fig. 174, B*), are broken up into columns by the strands of fibres passing from the white matter into the cortex. The medulla of white matter consists of medullated nerve fibres, which become non-medullated in the grey cortex.

In the sensory convolutions the large pyramidal cell layer is less marked.

If it be desired to study especially the ramifications of the nerve cells, a specimen stained by Golgi's process should be examined (see "Appendix"). On the other hand, Weigert's hæmatoxylin is more suitable for showing the strands of fine medullated nerve fibres running in the cortex. For showing the

characters of the nerve cells themselves in the different layers other stains are available, such as carmine, hæmatoxylin, bismark-brown, etc. One of the best is aniline blue-black, which seems to have a special affinity for nerve cells. The sections require to be left in it a considerable time (two or three days are often required), and may then be mounted in balsam.

Examine the following specimens :—

(1.) *V. S. Convolution of cerebrum (human), stained with aniline blue-black. B. (See Fig. 174, B.)*

Under the low power distinguish between the white matter forming the core of the convolution and the grey which covers it. The former is readily recognisable from the absence of nerve cells in it, and its fibrous appearance; the latter from the presence of the pyramidal cells, easily recognisable even under this power, and the molecular (as opposed to fibrous) character of its basis. Under the high power examine especially the different layers of the cortex, noting especially the characters of one of the large pyramidal cells. Observe its shape, the processes given off from it, the presence of a well marked nucleus and nucleolus, and the fact that the cell lies in lymph space.

(2.) *V. S. Convolution of cerebrum (human), stained with Weigert's hæmatoxylin. B.*

Study the specimen especially for the nerve fibres in the grey matter. Under the low power observe the core of white matter stained blue with the reagent in contrast to the somewhat yellowish brown of the cortex. Note, however, that the latter is somewhat modified by the numerous strands of fine medullated fibres in it revealed by the reagent. Put on the high power and observe these more carefully. The following description of their arrangement is given. They are absent immediately below the pia mater, but form a well marked layer parallel with the surface in the inner part of the outer layer. In the small pyramidal cell layer the nerve fibres cross each other in different directions. This is continued in the next layer, that of the large pyramidal cells, in the deeper part of which it results in the production of a well defined band of greater density. The large pyramidal cell layer also contains well marked strands of fine medullated fibres running radially, which are indicated in *Fig. 174, B.* As we pass inwards another condensation of the general network to form a band occurs in the lower part of the fourth

layer, and the radially running strands are still more marked, and the fibres forming them thicker. Finally, at the inner margin of the grey matter, the radial strands may be traced into the white matter of the core.

THE CEREBELLUM.

A vertical section of the convolutions of the cerebellum shows that, like those of the cerebrum, they consist of a central, white core or medulla invested with a grey cortex. The white core branches like a tree, and has hence been called the *arbor vitæ*.

At the surface is the pia mater (*Fig. 174, A, a*) from which capillary vessels pass into the cortex or grey matter. This consists of two layers, an outer broader one, the *molecular layer*, and an inner narrower, the *granular* or *nuclear layer*. Between these two is a layer of ganglion cells, named after Purkinje. The molecular layer is composed partly of a bed of neuroglia, partly of a network of branched nerve fibrils. By staining with Golgi's process small neuroglia cells are revealed, and also branched nerve cells. These are said to give off numerous branched peripheral processes, which pass through the molecular layer towards the surface, and a central axis cylinder process, which runs parallel with the surface above the cells of Purkinje, and gives off branches which form a kind of basket-work around the bodies of these cells. They have hence been termed "basket cells." Purkinje's cells occur in a single layer. They are large cells with a somewhat flask-shaped body, a nucleus and nucleolus, and give off one, or it may be two, chief peripheral branches, which rapidly break up to form an arrangement which is shown in *Fig. 174, A*, and also *Fig. 59, d*, and which, from its resemblance to the antlers of a stag, has caused the cells to be termed "antler cells." The axis cylinder process arising from the opposite central pole of the cell passes through the nuclear layer to join the fibres of the white core. The nuclear layer contains many small branched nerve cells, to which its appearance is for the most part due. Some of the nuclei, however, are those of neuroglia cells and blood-vessels. The nerve cells are stated to give off branches, which pass both into the molecular layer and the central core of white matter.

Numerous fine medullated fibres are to be found throughout the grey matter.

FIG. 173.

V.S. CEREBELLUM (HUMAN), STAINED WITH WEIGERT'S HÆMATOXYLIN
× 20.

- a.*—Core of white matter.
 - b.*—Nuclear layer
 - c.*—Molecular layer
 - d.*—Ganglion cells.
 - f.*—Pia mater.
- } grey matter

FIG. 174.

A.—V.S. CEREBELLUM (HUMAN), STAINED WITH PICO-CARMINE × 300.

- a.*—Pia mater.
 - b.*—Molecular layer.
 - c.*—Granular or nuclear layer.
 - d.*—White core.
 - e.*—Purkinje's ganglion cells (antler cells).
- } grey matter.

B.—V.S. CEREBRUM (HUMAN), STAINED WITH PICO-CARMINE × 100
(SEMI-DIAGRAMMATIC).

- a.*—Pia mater.
 - a.*¹—Molecular layer.
 - b.*—Small pyramidal cell layer.
 - c.*—Large " "
 - d.*—Layer of irregular cells.
 - e.*—Fusiform cell layer.
 - f.*—Layer of white matter.
- } grey matter.

Fig. 173.

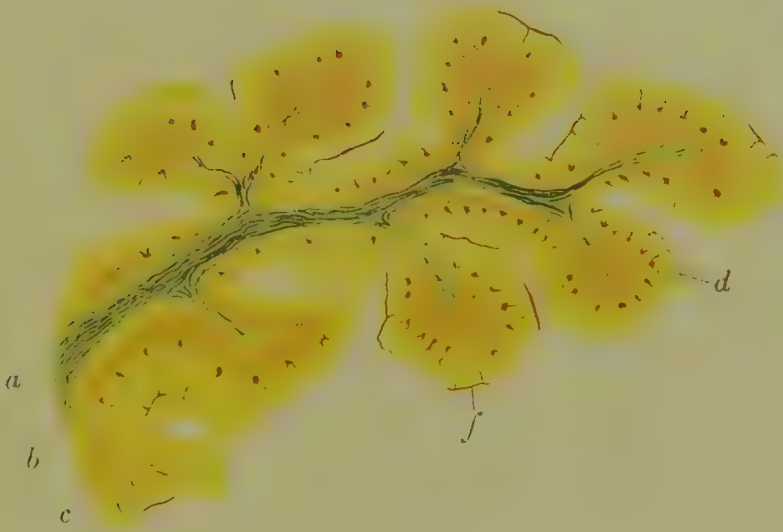
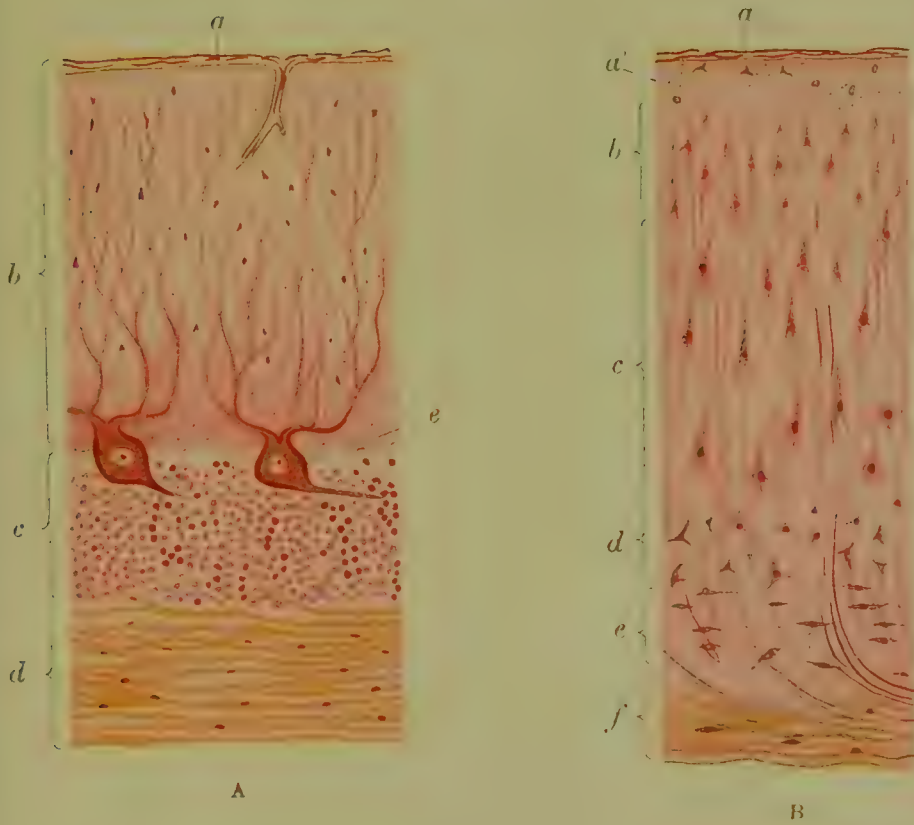


Fig. 174.



Sections of the cerebellum may be treated in much the same way as those of the cerebrum.

V. S. Cerebellum (human), stained with Weigert's hæmatoxylin or carmine. B. (Figs. 173 and 174.)

Under the low power (*Fig. 173*) study the general arrangement of parts. Note the central core (*a*) stained blue in a Weigert preparation; the granular or nuclear layer (*b*), and the molecular layer (*c*). Between the two last distinguish the layer of antler cells (*d*), the large, somewhat flask-shaped bodies of which are readily distinguishable under this power. At the surface note the pia mater (*f*) sending capillary branches into the substance of the cortex.

Under the high power (*Fig. 174, A*) study the features of the different layers as they may be seen in a specimen stained with carmine or hæmatoxylin. Note again the pia mater (*a*) at the surface, the broad molecular layer (*b*) beneath it, the antler cells (*c*), whose peripheral processes pass through the molecular layer towards the surface, the nuclear layer (*e*), and the layer of nerve fibres (*d*).

APPENDIX TO CHAPTER XVII.

METHODS OF PREPARATION.

The brain and spinal cord.—Use the central nervous system of man for the most part, but it is also well to take that of a small animal, such as the cat. In dealing with the medulla especially, a small cord possesses great advantages, as the sections of the human medulla are rather large, and one cannot get an unlimited supply of the tissue. The cord and brain should be removed whole by cutting through the laminæ of the vertebræ and round the vault of the skull with a pair of pliers or strong scissors. Of course, in the case of man and the larger animals the saw will have to be used. The proceeding is one requiring care, practice, and patience, as it is a point of its success to obtain the whole central nervous system in an uninjured condition. It is, perhaps, scarcely necessary to say that the animal must have been only recently killed, as the brain especially very rapidly undergoes changes. After removal, in the case of man and the larger animals, separate the spinal cord from the brain immediately below the decussation of the pyramids, make transverse incisions into it along its whole length at a distance of $\frac{1}{2}$ to 1 inch from each other, and suspend it in a tall glass jar or tube filled with Müller's fluid or bichromate of potassium or ammonium. A strong tube of some twenty inches in length, and one and a half in diameter, which can be fitted with a cork at the open end like a test-tube, is very convenient for the purpose, and it is well to have two or three of these of different sizes. If the cork is perforated with a wire ending in a hook, the cord may readily be suspended by a piece of thread in the centre of the tube. It should be kept in a cool place and in the dark, and be examined and handled from time to time, the fluid being constantly changed. It takes several weeks (four to six) to harden the cord properly in Müller's fluid or bichromate of potassium; in bichromate of ammonium the time required is still longer. The hardening may be completed by immersion in spirit for a few days, but if kept in spirit for a long time the tissue deteriorates.

The medulla and pons should be removed * from the rest of the brain by dividing the superior middle and inferior peduncles of the cerebellum, and

* It is sometimes preferable to incise the brain freely and place in Muller's fluid on tow in a jar for a day or two before performing the dissection, as the tissues are then handled with a little more safety, but in such a case the fluid must be changed on the second day, and the incisions must be such that the fluid penetrates the deeper parts, otherwise these will be found to soften.

the crura cerebri immediately above the pons Varolii. The medulla and pons after being then dissected out should be incised transversely at intervals of half an inch, and placed upon tow in a vessel containing a plentiful supply of one of the reagents already mentioned as being used for the cord. Now remove whatever parts are required of the cerebral and cerebellar convolutions. Small cubes of half an inch in diameter, showing the white and grey matter may be taken from the frontal, occipital, and temporo-sphenoidal lobes of the cerebrum, and one or two pieces of the cerebellum showing the arbor vitæ well; and placed upon tow in excess of the hardening fluid. As in the case of the cord, so with the medulla and brain, constant changing of the fluid and examination of the condition of the tissue from time to time is necessary. When the pieces are hardened sufficiently they should be resilient but not brittle; there should be no tendency to cracking or crumbling when they are pressed between the thumb and forefinger. If so they are not successful, and it is useless to try to make sections of them. If possible it is well to make use of this tissue immediately after hardening, as it does not improve with keeping, but if this is a necessity a solution of chloral hydrate of the consistence of thin syrup seems to do better than spirit. The tissue is first washed in water after being hardened, and then transferred to the chloral hydrate solution in which it is preserved. Before cutting in gum the pieces are placed in water for a few hours, so that the chloral is dissolved out of them, and they are then infiltrated with the freezing mixture in the usual manner.

It is often possible in dealing with the central nervous system of small mammals to harden the brain medulla and spinal cord together. For this purpose an earthenware box, twelve or more inches in length, by four or more in breadth, is convenient. The bottom can be covered with tow, upon which the brain and cord lie in an excess of the fluid, and a lid is easily made of sheet glass cut a suitable size. Such earthenware boxes with glass lids are useful for many purposes, particularly when a considerable quantity of tissue requires hardening, or an excess of fluid is essential. They have the advantage, too, of keeping their contents cool, and the light is very readily excluded.

The spinal cord.—*Preparation of the tissue for cutting sections.*—Harden in Müller's fluid or bichromate of potassium. The question of storage of tissue after hardening presents some little difficulty in the case of the spinal cord, though not so much as in that of the cerebrum and cerebellum. After keeping some time in spirit, the tissue tends to become brittle, and it is undoubtedly better to use it just after hardening has been completed. This has a particular advantage, too, in the case of sections to be stained with Weigert's hæmatoxylin, as they are already saturated with the chromium salt. If the tissue cannot be dealt with immediately after hardening, it may, as an alternative to spirit, be kept in a gum solution (such as is used for freezing), to which carbolic acid may be added in sufficient quantity to prevent souring. It may also (like the brain) be kept in a solution of chloral hydrate of the consistence of thin syrup. Finally, if it be intended to stain it with carmine, it may be stained in bulk in borax-carmine, saturated with paraffin in the usual way, and preserved in block form ready for cutting, indefinitely.

Cutting and staining.—The tissue may be cut in either gum, paraffin, or celloidin, the choice depending largely on how they are to be stained. As a general rule, gum is satisfactory if the pieces have been well hardened and are not brittle; this they should never be, as the sections are so readily broken in water, and in transference from one fluid to another.

The sections may be stained with the following reagents:—

(1.) *Weigert's hæmatoxylin.* Cut in gum, stain as directed on page 41, and mount in balsam. The medullary sheaths of the nerve fibres are stained blue, while the grey matter is left for the most part a brownish yellow (*Figs. 169–173*). Even here, however, the reagent reveals the presence of numerous very fine medullated fibres, which are not shown by other methods. This is undoubtedly the best method of staining the spinal cord. In sections of the medulla especially, where a rearrangement of the grey and white matter takes place, it is impossible to over-estimate the value of the method. It is certainly somewhat complicated in the number of processes involved, and some little difficulty is at first to be anticipated, but after the first few experimental failures, complete success is readily attainable. The great thing is to select one method, to stick to it, and endeavour to perfect it. It is a mistake to attempt to use all the different modifications frequently given.

(2.) *Aniline blue-black.* Sections of the cord cut in gum frequently do very well in this. The grey matter is picked out from the white, the nerve cells themselves, for which the reagent has a special affinity, being deeply stained (*Figs. 165–168*). It is thus very useful for sections of the cerebellum and cerebrum, especially the latter. The staining takes time, however. It is frequently necessary to leave the sections in the solution for three or four days, inspecting them from time to time. Mount as usual in balsam. It has this advantage over Weigert's method, that, when the sections have to be supplied in quantity to a class, not so many are lost by breaking up. Apart from the handling they undergo in Weigert's process, it tends of itself to make the sections much more brittle than before.

(3.) *Strong Carmine.* Sections do well in strong carmine, as far as the nerve cells are concerned; but the grey matter is not sufficiently differentiated from the white. Mount in balsam.

(4.) *Borax Carmine.* Pieces may be stained in bulk and cut in paraffin; the result is much the same as in staining sections in carmine; both give good high power studies of transverse sections of the white matter, and of nerve cells.

For teased and cover-glass preparations of spinal cord, *see* page 214.

Golgi's silver and sublimate methods for revealing cells of nerve tissue.—By these methods the cells and their processes are stained black, and the network formed by the latter becomes revealed in a very beautiful manner.

Silver method: The process may be carried out: (1,) slowly; (2,) at a medium rate; (3,) rapidly.

The slow method.—The pieces of the cord or brain (which should be cut quite small, $\frac{1}{8}$ cubes are quite large enough), are first of all hardened in potassium bichromate. From three to six weeks may be required, as the process should be conducted slowly, beginning with a 2 per cent. solution.

and increasing the strength gradually up to 5 per cent. Wash in distilled water for a few moments, and transfer to '5-1 per cent. silver nitrate solution, in which they may remain one, two, or three days. Then harden in alcohol; make sections by hand, and mount in balsam after clarifying. It is well not to apply a cover-glass, as sections keep better without it.

Medium method: Place pieces of the fresh tissue 3-5 days in 3 per cent. potassium bichromate, then 3-5 days in osmico-bichromate solution, viz. :—

Potassic bichromate	3 per cent.	20 c.c.
Osmic acid	1 „	5 c.c.

They may be kept in this for two to three days, and then transferred to silver nitrate solution '5-1 per cent. for one, two, or three days; then treat as before.

Rapid method: Place pieces of the fresh tissue in osmico-bichromate solution for two to three days, and then in silver for one to two days; then treat as before.

Golgi's method stains neuroglia and nerve cells and the processes coming from them black, so that a very complicated network is revealed. The blood-vessels are also usually stained black. For staining the network of peripheral or protoplasmic processes, the slow method is stated to be the best; for the axis cylinders and their processes the rapid and medium are to be preferred.

Sublimate method: Harden small pieces in potassium bichromate, and place in '25 per cent. solution (watery) of corrosive sublimate. Here they may remain for many weeks, the fluid being constantly changed. At the end of a fortnight some of the pieces may be tested by cutting hand sections. Mount in balsam as before, but do not apply a cover-glass. The sections should be thoroughly washed before mounting, to avoid the subsequent appearance of crystals. As in the case of the silver method, the nerve and neuroglia cells and the blood-vessels are affected. The parts "stained" are in reality white, their blackness by transmitted light being due to their opacity. Golgi recommends the washing of the sections for a minute or two in the following solution, whereby the white is converted to black. This is especially applicable where the object is to reveal the diffuse network of the central nervous system. The sections are washed in distilled water, immersed in the solution for a minute or two, again carefully washed, and mounted in balsam, without a cover-glass.

The following is the formula :—

A.	Water	1000 c.c.
	Hyposulphite of soda	175 grams.
	Alum	2 „
	Ammonium sulpho-cyanide	10 „
	Common salt	40 „

Filter at the end of a few days.

B.	Water	100 c.c.
	Chloride of gold	1 gram.

Take of A, 60 c.c.

B, 7 c.c. Mix.

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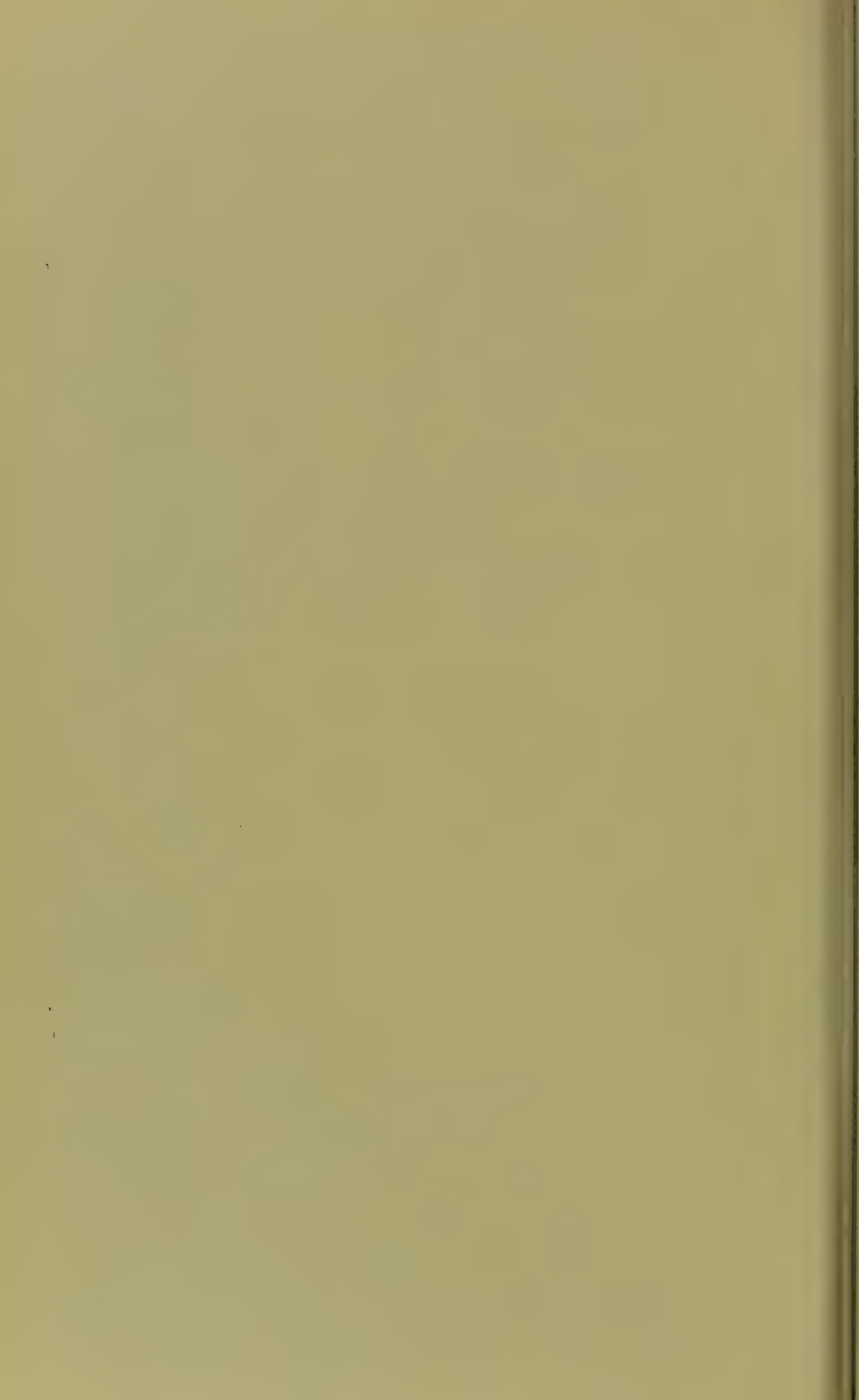
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